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RADIOGRAPHIC STUDIES ON THE FORMATION OF THE HEN'S EGG SHELL

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(*Received* 25 *August* 1950)

(With Plate 1 and Six Text-figures)

INTRODUCTION

This paper describes studies by means of X-ray photography on the shape, size and rate of formation of the hen's egg shell and on the movements undergone by the egg during shell formation. Although it is the only method by which a single egg shell can be directly observed at all stages of its formation, radiography appears not to have been used previously for this purpose.

The eggs of birds show a wide variety of shapes and sizes; some, such as those of owls and kingfishers, are almost spherical and others are almost perfect ellipsoids in longitudinal section. Most are, however, like the hen's egg, a little blunter at one end than at the other, though within each species, and particularly with the domestic fowl, there is considerable variation from one bird to another. Various ideas have been advanced regarding the significance of this difference of curvature between the two ends—a problem which has excited curiosity and comment from early times. In the case of the plover, it has been suggested that the eggs, arranged with their pointed ends inwards like the quadrants of a circle, can be most easily covered by the sitting bird, but many species survive equally well with less conveniently shaped eggs. In other cases the shape of the egg has been correlated with the dimensions of the embryo developing inside it. Some swimming birds, where the long, boat-shaped sternum has only a very feebly developed keel, have rather long, narrow eggs, for example, but such correlations are by no means universal.

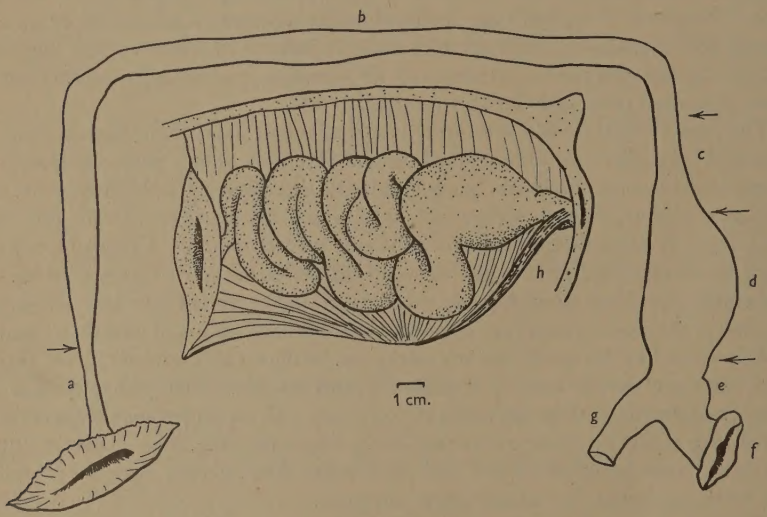
Although it is difficult, therefore, to determine the precise significance of the characteristic shapes of birds' eggs, it is of interest to inquire how this shape arises and to what extent the dimensions of the egg are determined by the forces involved in the formation of such a large and rigid object. Also of considerable interest are the rate and mechanism of the striking secretion of calcium carbonate during shell formation—5 g. per day, or about 2 g. per kg. body weight per day, in the hen—and, in other species, the mode of formation of the often complex patterns of shell pigmentation, though the latter problem has not been studied here.

Radiographic methods were first used in this work as a more reliable guide than palpation for determining the stage to which shell secretion had progressed, in order that shell glands could be examined histologically and chemically during various phases of their activity. However, the X-ray photographs proved to have considerable intrinsic interest because they constitute the only means by which it is possible

to follow, for a single egg: (1) the rate of shell deposition, (2) the changes in volume of the shell, and (3) the movements of the egg in the oviduct between the commencement of shell deposition and the time of laying. After a general survey of the radiographic observations, these three specific points are considered in turn.

MATERIALS AND METHODS

All the observations described here were made on a Rhode Island Red × Black Leghorn breed of hens used for domestic egg production. Birds selected at random during their first laying period were kept under close observation by means of trap-nests. To clarify the method of choosing birds for X-ray examination at suitable phases of the laying cycle, it is convenient to give a brief summary of the normal time relations of egg production.



Text-fig. 1. The oviduct of the fowl in the hypertrophied laying condition, shown both extended and *in situ* (suspended by its mesenteries). The approximate boundaries between the successive regions are indicated in the extended oviduct by arrows. The regions are labelled as follows: *a*, funnel; *b*, magnum, which secretes the albumen; *c*, isthmus, which secretes the shell membranes; *d*, shell gland, or 'uterus'; *e*, vagina; *f*, cloaca; *g*, intestine; *h*, muscular band. Both oviducts contain an egg in the shell gland.

The normal laying cycle. About 26 hr. elapse between the laying of successive eggs and this period is made up roughly as follows (Warren & Scott, 1935): ½ hr. between laying one egg and discharging the next yolk into the single left oviduct (Text-fig. 1); ½ hr. in passing into the mouth of the fimbriated funnel; 3 hr. in the magnum, where most of the albumen is secreted over the yolk; 1 hr. in the isthmus, where the tough shell membranes are formed; 20 hr. in the shell gland (the so-called 'uterus'), where the calcareous shell is secreted. These average figures were obtained by examining

a large number of hens; each was dissected at a known time after the laying of an egg and the point in the oviduct reached by the new egg recorded.

When in full lay, a hen usually produces eggs, one per day, for possibly 5 days, laying early on the first day and 1 or 2 hr. later each succeeding day (on account of the 26 hr. cycle described above). Hence on the fifth day an egg will be laid in mid-afternoon and when this happens, the next yolk is not ovulated until the following morning (sixth day). Accordingly, no egg is laid on the sixth day, but the cycle recommences with an egg laid early on the morning of the seventh day. Hens conform quite closely to this pattern during the spring peak of egg production, but during periods of less intense laying activity there may be considerable divergences.

Hens were usually taken for X-ray examination on the morning following a day on which they had not laid. This means that they were taken on the seventh morning of the cycle described above, i.e. the morning when the first egg of the new clutch will be laid, most commonly between 7 and 8 a.m. This time was noted and X-ray examination of the hen begun a few hours later, about midday, by which time the new egg, surrounded only by shell membranes, should be about to move into the shell gland. Once the egg had been detected, radiographs were taken every 2 hr., or sometimes more frequently, until about 9 p.m. and were continued at about 8 a.m. next morning until the egg was laid (usually about 10 a.m.). That part of shell secretion which goes on during the night is unavoidably missed, but by following an egg which is ovulated early in the day it is possible to trace the first half of the process (which proved to be the most interesting), together with the last few hours.

Radiographic methods. Radiographs were taken with the bird held so that it rested on the film cassette. For direct visual examination by means of the fluorescent screen, the bird was allowed to brood naturally in a small coop placed immediately in front of the screen. In the darkened room the hens soon settled quietly and could thus be examined at intervals of a few minutes over periods of several hours. The exposure to X-rays had no ill effects on general health, nor on egg production. It is of interest, however, to record that at the next moult one hen changed its feather colour over the whole body, from deep brownish black to pure white; it is, of course, unknown whether this was due to the previous irradiation.

The radiographs were taken with a Coolidge, rotating-anode X-ray tube of 1 mm. focal spot, using an exposure of $\frac{1}{20}$ sec. at 60 kV. and 100 mA. and a distance from tube to object of 40 in. The time of development (usually 7 min., never less) and the temperature of the developer were kept constant throughout the processing of any one series of radiographs.

Nomenclature. In order to avoid confusion, the terms used with reference to the egg are noted here. 'Leading' and 'trailing' are used of the egg regarded as a moving object. 'Cranial' and 'caudal' are used to denote ends of the egg nearest the hen's head and tail respectively, i.e. in the customary anatomical sense. The terms anterior and posterior are avoided, since in the past there has often been doubt as to whether 'anterior end' referred to the leading end of the moving egg, or to the end nearest the hen's head.

RADIOGRAPHIC OBSERVATIONS

About 6 hr. after the last egg has been laid, the new egg shell can be first detected in a radiograph (Pl. 1, fig. 1) as a faint line crossing the pubis near its distal extremity and again higher up the bone, 2–3 cm. below the acetabulum. The egg lies immediately beneath the vertebral column, with the pelvis, shaped like an upturned boat, arching over it. From its posterior position, the egg must already be in the shell gland, the last major region of the oviduct (Text-fig. 1). In successive radiographs taken later during the process of shell deposition (Pl. 1, figs. 1–6), the egg shadow becomes steadily brighter and measurements of the relative brightness with a densitometer have been used to determine the rate of deposition of mineral matter in the shell, as described in a subsequent section.

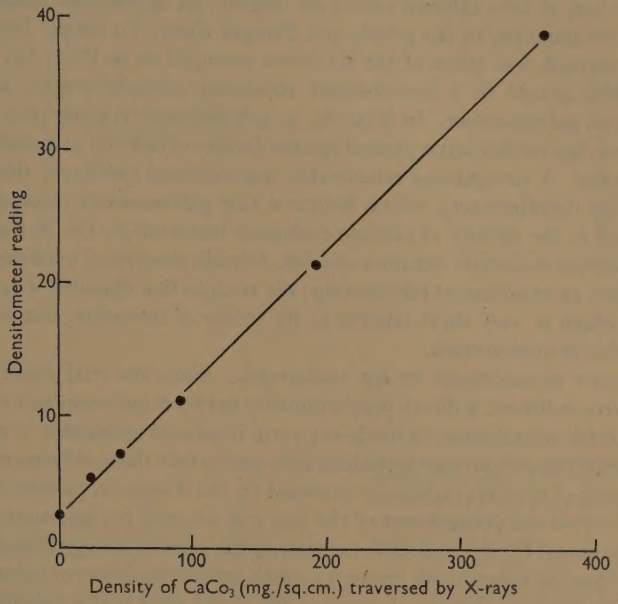
The period passed in the shell gland in these experiments was 20 ± 1 hr. During this time the egg is more or less stationary; it certainly is not being squeezed along the oviduct by the action of peristalsis in the manner envisaged by D'Arcy Thompson and others. There is a small, but easily measurable, increase in egg volume during the early stages of shell deposition, but this swelling ceases when the egg has been in the shell gland for a few hours—at the moment when, as shown later, the rate of shell deposition begins to increase rapidly.

The characteristic shape of the egg is clearly discernible as soon as the egg itself can be detected in a radiograph (Pl. 1, fig. 1). The pointed end lies caudal throughout most of the time which the egg spends in the shell gland, but in nearly all cases the egg undergoes a 180° rotation about its short vertical axis (Pl. I, fig. 5) about an hour before it is laid, so that the blunt end is now posterior and emerges first when the egg is laid. During the act of laying, which has been observed on the fluorescent screen, but not photographed, the egg is not moved by peristalsis as in the passage of a food bolus along the alimentary canal; instead the whole shell gland with contained egg is prolapsed through the cloaca to the exterior and then the gland is withdrawn into the hen, leaving the egg outside. The new-laid egg has no air space, but within an hour or two of being laid the egg has developed an air space as a result of cooling and evaporation.

THE RATE OF SHELL DEPOSITION

One method of measuring the rate of shell deposition is to take a large number of birds, kill them at different times after the last egg has been laid, extract the new egg from the shell gland and weigh its shell and, finally, plot all these points on a graph of weight against time. Each point is very accurate for a particular hen, but no two points refer to the same hen, so that the curve produced is of limited significance. If, however, it could be shown that the brightness of the egg shadow in a radiograph bore some simple relation to the amount of mineral matter in the shell at any stage, then it would be possible to obtain successive measurements of mineral density on the *same* shell throughout its deposition and hence to construct a curve, all the points on which would be strictly comparable since they would relate to the same egg. This approach proved to be possible experimentally using series of radiographs of the kind shown in Pl. I, figs. 1–6. The steps involved are set out below.

Tests on a graded series of calcium carbonate suspensions. The first step was to take a radiograph of a graded series of calcium carbonate suspensions (since 94% of the weight of the shell is $CaCO_3$), in order to determine how photographic density in the radiograph varies with the density of calcium carbonate through which the X-rays have passed. For this purpose, different weighed amounts of calcium carbonate were placed in each of five small Perspex boxes and a known, constant volume of a 10% gelatin solution pipetted into each and into a sixth box containing no calcium carbonate. The contents were stirred thoroughly and made to gel rapidly (at 2° C.) to preserve the even distribution of calcium carbonate in the first five boxes. The final depth of the suspension was about 15 mm. The appropriate range of calcium



Text-fig. 2. The linear relationship between the calcium carbonate content of six gelatin gels and the densitometer readings on a radiograph of the gels (Pl. I, fig. 7).

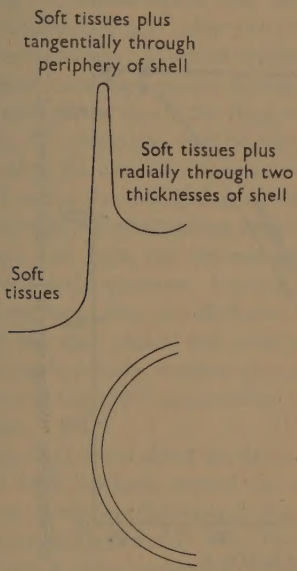
carbonate concentrations for this standard series was determined in the following way. Assuming the surface area of the egg approximates to that of a prolate spheroid with the same major and minor axes as the egg and knowing the weight of calcium carbonate which is distributed fairly evenly over this surface, it is possible to calculate the density of calcium carbonate (g. per sq.cm.) traversed by X-rays passing *radially* through the shell. Then from the geometrical relationships shown in Text-fig. 4 it is possible to calculate the much greater density of calcium carbonate traversed by the X-rays passing *tangentially* through, for instance, the pointed end of the shell. The suspensions of calcium carbonate varied from zero up to a maximum density slightly above the density traversed by the X-rays passing tangentially

through the shell, so that the standard graded series should straddle precisely that range of calcium carbonate densities to be expected in the developing shell. Hence conclusions drawn from the standard series about the relation between photographic density and the calcium carbonate density in the object can justifiably be applied to egg radiographs. The resemblance between the two is further increased by the fact that the radiographs of the developing shell and of the standard calcium carbonate suspensions both contain, beside calcium carbonate, a certain additional amount of absorbing matter—the feathers and soft tissues of the hen in one case and the Perspex and gelatin in the other. These account for the plateau labelled 'soft tissues' in Text-fig. 3, and for the fact that the line in Text-fig. 2 does not pass through the origin, but has, at zero calcium carbonate density, an appreciable transept on the ordinate corresponding to the gelatin and Perspex alone. To obtain Text-fig. 2, an X-ray photograph was taken of the six boxes arranged as in Pl. 1, fig. 7, and the negative then placed in a densitometer consisting of light source, narrow slit, photocell and galvanometer. In Text-fig. 2, galvanometer reading (not percentage transmission, nor extinction) is plotted against density of calcium carbonate traversed by the X-rays. A straight-line relationship was obtained (whatever the sensitivity setting of the densitometer), which indicates that galvanometer reading is directly proportional to the density of calcium carbonate traversed by the X-rays, over the range of calcium carbonate densities studied. Certain theoretical considerations lead one to expect an exponential relationship; the straight line obtained may, therefore, be an arc which is very short relative to its radius of curvature, but for practical purposes this is unimportant.

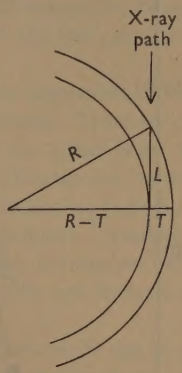
Densitometer measurements on egg radiographs. Since the trial experiments described above indicated a direct proportionality between galvanometer reading and density of calcium carbonate in the X-ray path, it seemed reasonable to make densitometer measurements on the egg radiographs and to take these as being proportional to the density of calcium carbonate traversed by the X-rays in passing through the shell. The tip of the pointed end of the egg was selected for measurement for the following reasons: (1) it is desirable to measure the edge of the egg image, where the X-rays are passing tangentially through a much greater thickness of calcium carbonate than elsewhere, so that the absorption due to the shell is at a maximum relative to the general background absorption; (2) for similar reasons it is best to measure at the pointed end of the egg, which, up to within an hour or two of laying, lies posterior in the shell gland, where the surrounding soft tissue are thinnest; (3) the tip of the pointed end is, in any case, the place which can be most accurately selected each time, to ensure that the measurements are comparable. As shown in Text-fig. 3, the radiographs of laying hens were scanned over about 3 mm. on each side of the egg-shell boundary; in critical regions (i.e. the peak of the curve in Text-fig. 3) measurements were made every 0·05 mm., the width of the beam of light used in the densitometer being also 0·05 mm.

Such scans have three main components (Text-fig. 3): a low plateau representing the absorption of the soft tissues round the egg; a higher plateau representing soft tissues plus two thicknesses of shell; between these, a peak representing the region

where X-rays are passing tangentially through the shell. By making scans across the pointed end of the shell in each of a succession of radiographs taken at intervals during the formation of a single shell (e.g. 6, 9, 11, 13, 23 and 25 hr. after the previous egg had been laid) a series of such curves was obtained. In the earliest curves, the edge of the shell was represented only by a low hump, but the height of the peak increased progressively in the later radiographs, gradually assuming the form shown in Text-fig. 3, which is a typical scan across the edge of the shell in a 23 hr. radiograph. In each case the height of the peak above the low and relatively constant



Text-fig. 3. The general shape of a densitometer scan over the edge of the egg-shell image in a radiograph of a laying hen. For convenience of representation, the dimensions have been somewhat distorted; the radius of curvature of the shell should be much greater in proportion to the thickness of the shell.



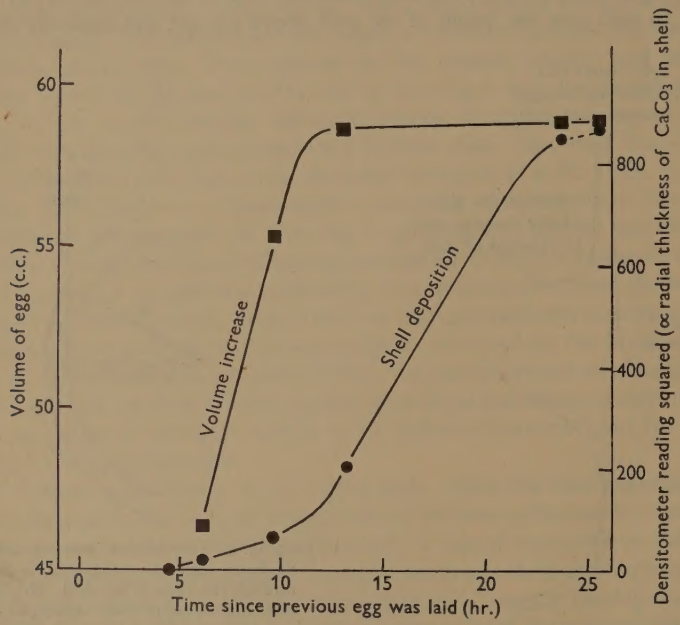
Text-fig. 4. The relation between radial shell thickness and the tangential X-ray path through the edge of the shell. R= radius of curvature of outer shell surface, $2L$= maximum tangential X-ray path, T= thickness of shell. By Pythagoras,

$$L^2 = R^2 - (R-T)^2$$
$$= R^2 - R^2 + 2RT - T^2$$
$$\simeq 2RT \quad \text{(since } T^2 \text{ is small)},$$

therefore $T = \frac{L^2}{2R}$ or $T \propto L^2$.

soft tissue plateau was taken as a measure of the density of calcium carbonate traversed by the X-rays taking the longest tangential path through the shell (L in Text-fig. 4). From Text-fig. 4 it can readily be seen that the radial thickness (T) of the shell is closely proportional to the square of the tangential X-ray path (L). Hence, by squaring the heights of the peaks in the densitometer scans, it is possible to obtain a series of values, each of which is proportional to the radial shell thickness at a particular time during shell development. These squared values can then be plotted against time to show the rate of deposition of mineral matter in the shell (Text-fig. 5).

At the time when the last radiograph was taken ($25\frac{1}{2}$ hr. after the previous egg had been laid), the new egg had rotated, bringing the blunt end caudal and putting the pointed end in among heavily absorbing tissue (Pl. 1, fig. 6). Hence the last point in Text-fig. 5 was obtained by scanning across the blunt end of the shell and then applying a correction equal to the ratio (height of peak for pointed end/height of peak for blunt end) determined on the same egg after it had been laid.



Text-fig. 5. The rate of deposition of mineral matter in the shell and the rate of increase of volume of the egg while in the shell gland. Since $T \propto L^2$ (Text-fig. 4), each point on the shell-deposition curve is obtained from the corresponding densitometer scan, by squaring the height of the peak above the soft-tissue plateau (Text-fig. 3).

Detailed studies of this kind have been made on four hens and all gave similar S-shaped curves for the rate of shell deposition; one such curve is shown in Text-fig. 5. Shell deposition commences about 5 hr. after the previous egg has been laid. There is then an initial slow phase lasting about 5 hr., followed by a much more rapid phase, which lasts about 12 hr. Finally the curve flattens off in the last few hours before the new egg is laid, in accordance with the finding that abnormally long retention of the egg in the shell gland does not result in a substantial increase in shell weight. The possible significance of the S-shaped curve will be discussed later. Whatever its cause, the marked acceleration which occurs about 5 hr. after the onset of shell deposition has interesting repercussions on the osmotic swelling of the egg which will be described in the next section.

THE VOLUME AND SHAPE OF THE EGG

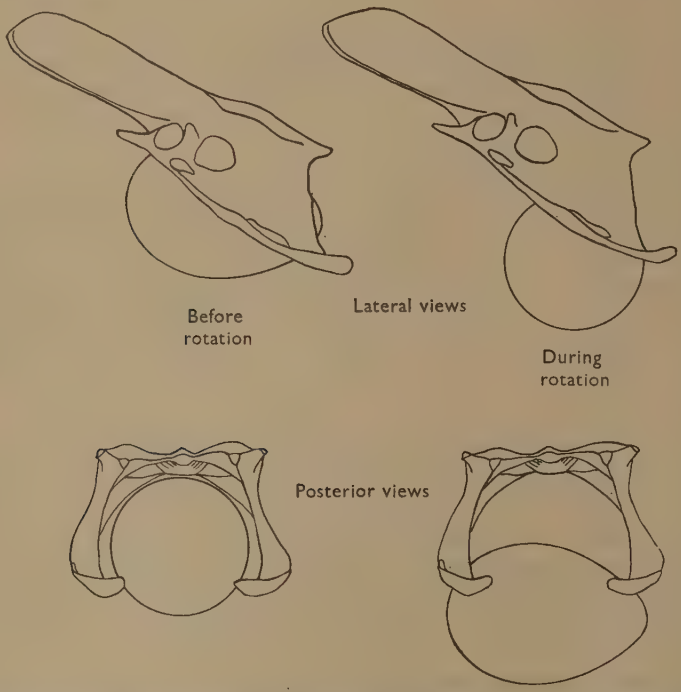
Measurements of the major and minor axes of the egg (in lateral and dorsoventral radiographs) shows that during the first 6 hr. spent in the shell gland the egg undergoes a small but appreciable increase in volume. In order to calculate its volume at various stages, the egg has been considered as a blunt half and a pointed half and these were assumed to be halves of two perfect prolate spheroids with identical minor axes, but different major axes. The values obtained for the volume are plotted against time in Text-fig. 5, which shows that from the time when this egg was first detected radiographically ($6\frac{1}{2}$ hr. after the previous egg had been laid) it underwent a 25% increase in volume. It is also evident that this increase in volume—presumably due to osmotic swelling—occurs during the early phases of shell deposition and ceases fairly abruptly at about the time where the rate of shell deposition begins to rise sharply, i.e. where the shell becomes rapidly more rigid and less permeable, about 12 hr. after the last egg was laid.

As it enters the shell gland, the egg consists of the yolk suspended by chalazae in the albumen, the whole being enclosed in the shell membranes. These are permeable to water and salts, but impermeable to proteins and the other large molecules abundant in the albumen. It is not surprising, therefore, that such a 'membranous' egg, if removed from the shell gland and placed in a fluid similar in composition to that in the shell gland, will swell to twice its original volume. Similarly, *in situ*, considerable volume increase goes on, but at a rate which diminishes rapidly until reduced to negligible proportions by the increasing rigidity and impermeability of the shell.

Little can be said about the factors determining the shape of the egg as no experimental work has been carried out on this subject. A few simple observations will, however, serve to refute much that has been said in the past on this problem—in particular the theory of D'Arcy Thompson for explaining the shape of the egg as the direct result of the peristaltic forces which propel the egg along the oviduct. These forces, it has been suggested, cause the formation of a blunt leading end (caudal in the hen) and a pointed trailing end (cranial in the hen), exactly as when a food bolus is propelled along the intestine by the contraction of circular muscles behind it and the relaxation of those in front (Gunther, 1792; Ryder, 1893; D'Arcy Thompson, 1908, 1942). However, all direct investigations of the orientation of the egg in the oviduct, either by dissection or, as here, by radiography, have shown that exactly the opposite is true. As soon as the two ends can be distinguished, it is found that the caudal, or leading, end is pointed and that the cranial, or trailing end is blunt. Moreover, prolonged study with the fluorescent X-ray screen shows that, apart from small oscillations about its axes and the rotation described below, the egg is stationary in the oviduct throughout the 20 hr. period of shell secretion.

A so-called 'membranous' egg, devoid of a calcified shell and enclosed only in the shell membranes, has a well-defined shape closely similar to other eggs laid by the same hen. Whatever factors determine egg shape must, therefore, operate in the isthmus where the shell membranes are secreted. Examination of freshly dissected

oviducts (in the greatly hypertrophied, laying condition) indicates that one important influence in determining egg shape is the fact that the end of the isthmus adjacent to the shell gland tends to be more contractile and more like a sphincter than the end adjoining the magnum, or albumen-secreting region of the oviduct. Since the egg greatly distends the narrow isthmus, it is to be expected that the caudal end of the egg, situated in the more contractile part of the isthmus, will become more pointed than the cranial end. However, although in accordance with the available evidence, this suggestion cannot be regarded as proved and the problem remains without a clear-cut solution.



Text-fig. 6. Diagrams to illustrate the way in which the egg sinks to a more ventral position during the 180° horizontal rotation which it undergoes shortly before being laid. As a result, the ends of the rotating egg, enclosed within the shell gland, swing well clear of the ischia and pubes.

THE ROTATION OF THE EGG

During the major part of shell secretion the egg undergoes only small oscillations, which coincide with, and are probably caused by, the breathing movements. There is slight pitching and yawing and probably also rolling; the latter could hardly be detected on the fluorescent screen, but the spiral markings on some egg shells suggest that it occurs. There are no large-scale movements until about an hour before the

egg is due to be laid, when it is rotated through 180° in a horizontal plane. Pl. 1, fig. 5 shows an egg which has completed half the rotation and is hence seen end-on in lateral view. This radiograph is slightly blurred because in order to observe the rotation the hen must be screened continuously and a 'still' had to be taken with the less intense beam used for screening, which necessitated an exposure of $\frac{1}{5}$ sec. (instead of the usual $\frac{1}{20}$ sec.); this was long enough for the normal visceral movements of the hen to blur the resulting radiograph. The fact that the egg rotates is also made clear by a comparison of Pl. 1, fig. 4 ($23\frac{1}{2}$ hr. after the previous egg was laid), where the *pointed* end of the new egg is caudal, with Pl. 1, fig. 6 ($25\frac{1}{2}$ hr. after the last egg was laid), where the *blunt* end of the new egg is caudal.

While being screened, the hen is sitting in a nest box, but during the actual rotation, which lasts 1–2 min., the bird rises partly to its feet. This may be related to the fact that during rotation the egg sinks to a more ventral position. Throughout most of shell secretion, the egg lies high in the body cavity in a mid-dorsal position immediately beneath the vertebral column, but the length of the egg is often equal to or greater than the maximum distance between the pubic bones and it is not surprising, therefore, that the egg enclosed in the shell gland sinks ventrally to a position where the ends of the egg will swing well clear of the pubes as the egg rotates. This point is illustrated diagrammatically in Text-fig. 6 and the radiographic evidence may be seen by comparing the position of the egg relative to the pelvis in Pl. 1, figs. 4, 5. The characteristic structure of the bird's pelvis—shaped like an upturned boat, open ventrally and with no trace of a pubic symphysis—facilitates this movement of shell gland and egg to a more ventral position, but it is more than a mere passive sinking of the viscera under their own weight as the hen rises to its feet, since normally the shell gland is held high in the body cavity even when the hen is standing.

DISCUSSION

The rate of shell deposition. The S-shaped curve obtained for the rate of shell deposition is in general agreement with the results of Burmeister, Scott & Card (1939), who studied sixty-seven eggs extracted manually at various times during shell secretion and plotted shell weight against time spent in the shell gland (determined by palpation). Their composite curve showed a small but appreciable change in slope after the egg had been in the shell gland about 3 hr. Direct weighing of the shell is a more accurate means of determining the stage of shell secretion than the radiographic estimation of mineral density used here, but it seems highly probable that this initial advantage is lost in the inaccuracies resulting from timing by palpation, and from the fact that no two points refer to the same egg.

No full explanation of the S-shaped curve can be given without a much fuller study of the physiology of the secretion process. One possible cause is that at first only a few cells respond to the secretion stimulus, whether nervous or hormonal, and hence the slow initial phase; but as more and more cells respond the rate rises and becomes a maximum when all the cells are secreting. It must also be remembered, however, that the innermost layer of the shell, the so-called mammillary layer which is secreted first, consists of a single thickness of small spheroidal particles of calcite (the

mammillae) and thus differs from the spongy layer which forms the main bulk of the shell. Hence it may be that the initial slow phase and the subsequent rapid phase are associated respectively with the secretion of these two distinct components of the shell, the mammillary layer and the spongy layer.

The volume of the egg. The close correlation between the time at which the rate of shell deposition increases and the time at which osmotic swelling is brought to an end has been mentioned already. The swelling of the egg is facilitated not only by the fact that the rate of shell deposition is slow during the first 6 hr. spent in the shell gland, but also by the fact that the first mineral matter deposited is in the form of discontinuous particles—the mammillae—which impede passage of fluid much less than would the same amount of material disposed in an even continuous layer over the surface of the egg. The small spaces between the mammillae are probably due to this expansion in surface area which the egg undergoes while they are being formed.

The increase in volume is due almost entirely to uptake of water and inorganic salts, especially potassium and bicarbonate ions (Beadle, Conrad & Scott, 1938; Burmeister, 1940). It involves hardly any increase in the nitrogen content of the albumen, which indicates that very little protein is absorbed. Scott, Hughes & Warren (1937) have shown, for instance, that if the second egg of a clutch is removed while still in the isthmus (i.e. before the 25% increase in volume which occurs in the shell gland), its albumen already contains 96% of the nitrogen present in the albumen of the previous egg, but weighs only half as much as the albumen of the previous egg. The extensive results of Pearl & Curtis (1912) point to exactly the same conclusion. This additional 10–15 ml. water, absorbed osmotically, dilutes the outer zone of the albumen producing the 4–5 mm. layer of thin albumen next to the shell, which can be clearly distinguished in a hard-boiled, new-laid egg. It also forms an important part of the reserves for the future development of the chick embryo inside its cleidoic egg.

The orientation of the egg in the oviduct. The discovery by Purkinje (1825) and von Baer (1828) that the hen's egg lies in the shell gland with its pointed end caudal indicated that there must be some sort of rotation shortly before laying, since it had been noted from very early times that the egg is usually laid blunt end first. There has, however, been much confusion on this subject and the actual rotation appears not to have been observed before.

The fact that the blunt end usually emerges first was known to Aristotle, and was confirmed by a large number of nineteenth-century observers listed in the extensive review by Bartelmez (1918). Nathusius (1895), for example, cites careful observations by Ernst on a series of forty-eight eggs, all of which were laid blunt end first. Wickmann (1895) studied the laying of eight hens, and although one of them laid five eggs consecutively with pointed end first, the majority emerged blunt end first. In the work described here, of ten eggs which were observed radiographically at frequent intervals until just before laying only one failed to rotate and this was in a restive hen, which had constantly to be adjusted for screening and may thus have been disturbed. Olsen & Byerly (1932) claim to have observed 1948 eggs at the precise moment of laying and state that only 17–34% (according to the breed)

emerged blunt end first. Their method of study was to wait till the bird rose to its feet, apparently to lay, and then either to remove the bird from the nest and let it lay in the observer's hand, or else to place one hand under the cloaca and grasp the egg as it emerged. In view of this it is significant that in the present work, where the hens were allowed to rest undisturbed in a nest box in a darkened room, they always rose to their feet at the moment when the egg rotated and then settled again before finally rising to lay the egg, perhaps an hour later. Hence it may well have been that many of the birds observed by Olsen & Byerly were disturbed at the moment of rotation of the egg and, as a result, dropped their eggs prematurely without turning them so that the blunt end became caudal.

Because eggs are usually laid blunt end first, Gunther (1792), von Hemsbach (1851) and Ryder (1893) assumed that they also passed down the oviduct with the blunt end leading. After making several assumptions, they were able to work out a complete theoretical picture of how the propulsive muscular pressure in the oviduct caused a tapering of the trailing (cranial) end of the egg, so that as the shell hardened the cranial end would become pointed and the caudal (leading) end blunt—which would in turn be in accordance with their original datum that the egg emerged blunt end first. This error was perpetuated by D'Arcy Thompson (1908, 1942) and J. A. Thompson (1923). In the account given in 'Growth and Form' of the mechanical factors involved in the formation of the hen's egg there are the further misstatements that the oviduct is only a few inches long, that the egg is only 10–12 hr. on its course down the oviduct and that the shell is secreted rapidly and at a late stage. In fact, the oviduct of a laying hen may be between 15 and 35 in. long, with an average of 25 in., and the egg spends some 25 hr. in the oviduct, of which 20 hr. are occupied by shell secretion. All investigators who have examined the orientation of the egg in the oviduct agree that until just before it is due to be laid the egg lies with its pointed end caudal—exactly the opposite of what is demanded by the peristaltic theories. This is true not only for the hen (Purkinje, 1825; von Baer, 1828; Coste, 1847; Taschenburg, 1885; Olsen & Byerly, 1932), but also for a hawk (Kutter, 1878), the pigeon (Blount, 1909; Patterson, 1909), the canary and several other birds (Wickmann, 1895). Purkinje and von Baer noted, however, that if a hen is killed near the time of laying, either end may be caudal, suggesting that rotation of the egg has occurred in some cases. The present experiments entirely confirm these conclusions—and extend them in that continuous observations have been made on the same hen and the actual rotation followed.

The mechanism of the rotation is unknown. One of the simplest methods of explaining it would involve the action of bands of muscle encircling the shell gland in a number of vertical planes equally spaced round the 360°. Then if the long axis of the egg were lying initially north and south, the contraction first of the north-west–south-east band of circular muscle, followed in clockwise order by all the other bands would rotate the egg clockwise in a horizontal plane. However, this suggestion cannot be checked until the histology of the shell gland has been investigated much more thoroughly.

It has been suggested that the need for the rotation arises from the way in which

the pointed end of the egg becomes inserted in a blind diverticulum of the shell gland, so that under muscular pressure it must rotate before it can pass to the exterior. There is no direct proof of this, however, and the fact that the shell gland is such a flexible muscular sac that it can be completely everted like a nemertine proboscis when the egg is laid strongly suggests that, if the pointed end were to become lodged in a diverticulum, it could easily be brought opposite the external orifice again by appropriate muscular contraction. The need for rotation could be more readily understood if the egg, after being rotated, were laid by peristalsis—it is for instance easier to propel an egg by exerting digital pressure on the pointed end rather than on the blunt end. However, the mechanism of egg-laying bore no resemblance to peristalsis in the hens examined here; instead, the entire shell gland was prolapsed through the cloaca and then withdrawn again, leaving the egg outside. It is difficult to see why such a manœuvre is more easily accomplished with the blunt end caudal than with the pointed end caudal.

Although the need for rotation is not understood, the general nature of the process shows interesting parallels with the changes in orientation sometimes undergone shortly before birth by the human embryo. It is noteworthy that in some mammals (man and guinea-pig) the embryos are so large relative to the pelvis that a special hormone (relaxin) is necessary to loosen the connective tissue of the pubic symphysis and increase the effective diameter of the pelvic girdle, shortly before birth (Hisaw, 1926; Catchpole, 1949). In the hen, on the other hand, the pelvis is widely open ventrally and presents no hindrance to the laying of the egg, nor to the ventral sinking of the shell gland, which ensures that the ends of the rotating egg swing well clear of the pelvis. A pubic symphysis is found among birds only in *Archaeopteryx* and *Struthio*. The main course of avian evolution has produced a pelvis exactly the reverse of the original vertebrate pelvis, which was a flat plate (or pair of plates), incomplete dorsally. In all birds, the broad dorsal expansions of the pelvis are immovably joined to the vertebral column over many segments (thoracic, lumbar, sacral and caudal vertebrae) and in all except *Archaeopteryx* and *Struthio* the girdle is widely open ventrally, thus producing a structure which is well suited both to withstand the shocks associated with landing after flight and to permit the visceral movements involved in the laying of large, hard-shelled eggs.

SUMMARY

1. Radiographic methods have been used to study the rate of deposition of the hen's egg shell and the changes in volume and orientation undergone by the egg in the shell gland.

2. Shell deposition commences about 5 hr. after the yolk is ovulated and several series of radiographs were obtained tracing the process from these earliest stages through to the fully calcified shell.

3. From radiographs of calcium carbonate-gelatin mixtures it was found that, for a series of comparable objects differing only in calcium carbonate content, the densitometer readings on their radiographs were directly proportional to the density of calcium carbonate traversed by the X-rays in each object.

4. Hence, densitometer measurements on the periphery of the shell in each of a series of radiographs taken during the development of a single egg shell give values which are proportional to the density (or thickness) of calcium carbonate traversed by the *tangential* rays. It is shown that the *radial* thickness is closely proportional to the *square* of these values.

5. Plotting these squared densitometer readings against time indicates that the rate of deposition of mineral matter in the shell follows an S-shaped curve, with a marked acceleration in shell deposition 5–6 hr. after its onset.

6. During its first few hours in the shell gland, the egg undergoes a 25% osmotic increase in volume. This swelling is brought to a fairly abrupt halt by the increase in the rate of shell deposition and the consequent increase in the impermeability and rigidity of the shell.

7. Throughout all but the last hour or two of its 20 hr. stay in the shell gland, the egg lies with its pointed end caudal. Shortly before it is laid, however, it usually undergoes a 180° rotation in a horizontal plane. Thus the blunt end finally becomes caudal and emerges first when the egg is laid. During the rotation, the egg sinks to a more ventral position. This is necessary because, in most hens, the length of the egg plus the thickness of the walls of the shell gland is greater than the width of the pelvis.

8. The possible significance of the S-shaped curve of shell deposition is discussed. The volume, shape and orientation of the egg are considered in relation to the needs of the chick embryo and to the characteristic structure of the bird's oviduct and pelvis.

I am much indebted to Prof. J. Gray, in whose laboratory most of this work was carried out, to Dr M. M. Swann, for advice and information, and to the Agricultural Research Council for a research studentship during the period 1945–7. This work would have been impossible without the collaboration of Mr J. A. F. Fozzard of the Department of Anatomy, who took the radiographs and was awarded the Royal Photographic Society's Rodman Medal for them.

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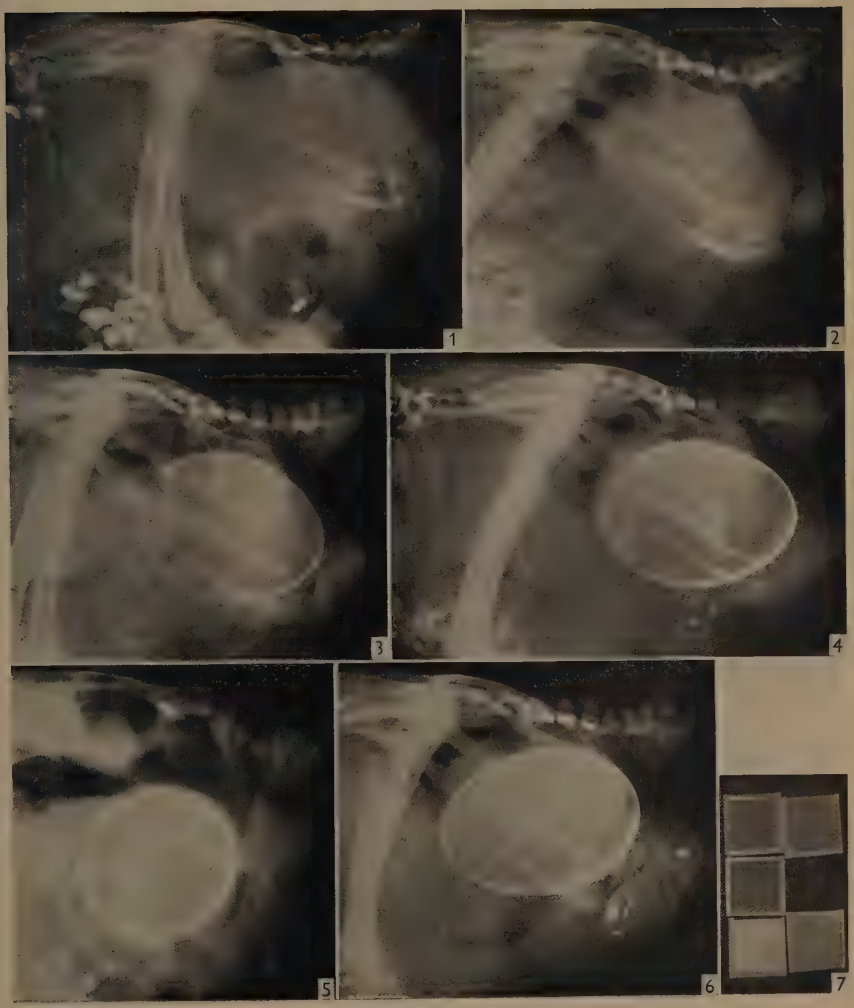
EXPLANATION OF PLATE 1

Fig. 1. Radiograph of a hen 6½ hr. after an egg had been laid. The new yolk has, by this time, received both albumen and shell membranes and has passed into the shell gland. The first faint outlines of the calcareous shell can just be detected.
Fig. 2. The same egg 9½ hr. after the previous egg had been laid.
Fig. 3. The same egg 12 hr. after the previous egg had been laid.
Fig. 4. The same egg 23½ hr. after the previous egg had been laid. The gradual increase in X-ray absorption by the developing shell is evident, but from mere inspection it is impossible to decide whether or not the rate of increase is linear. As shown in Text-fig. 5, the rate of deposition was, in fact, found to follow an S-shaped curve.
Fig. 5. A different egg which was screened at frequent intervals during the last few hours before it was due to be laid, so that the rotation was observed. This radiograph was taken half way through the rotation, when the long axis of the egg had moved through 90° in a horizontal plane and lies across the hen. The egg is therefore seen end-on for a minute or so before the rotation is completed. This radiograph is slightly blurred by the breathing movements, since, with the weaker beam used for screening, it was necessary to use an exposure of ½ sec.
Fig. 6. The same egg as in figs. 1–4, 25½ hr. after the previous egg had been laid (and half an hour before it was itself laid). In the preceding radiographs of this egg, the pointed end is caudal, but here the egg has rotated through approximately 180°, bringing the blunt end caudal.
Fig. 7. A radiograph of six gelatin gels. Five of these are in Perspex boxes and contain varying densities of calcium carbonate, up to and including the density traversed by the X-rays passing tangentially through the fully calcified shell. The sixth gel (right centre) contains no calcium carbonate (and is in a box with Perspex bottom, but very transparent sides, which are not visible here).



BRADFIELD—RADIOGRAPHIC STUDIES ON THE FORMATION OF THE HEN'S EGG SHELL

THE TEMPERATURE-PULSE RATE CURVE OF THE ISOLATED FROG'S HEART (*RANA TEMPORARIA*)

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(With Eleven Text-figures)

A seasonal difference in the temperature-pulse rate curve of the isolated frog's heart was described by Barcroft & Izquierdo (1931). In the summer they found an almost linear relation between temperature and frequency of pulsation from 5 to 20° C., while in the winter the relation was exponential over the same temperature range. Carter (1933) investigated the effects of various endocrine and related substances on the form of the temperature-pulse rate curve. He found that the addition of thyroxine to the medium perfusing the heart transformed the curve given by the winter heart into the summer form, but had no effect on that of the summer heart. He concluded that the seasonal change in the temperature effect on heart rate was controlled by the endocrine system, and that the thyroid by increase in its activity in the summer was the effective agent.

Although the change of frequency with temperature shows this seasonal difference, no experiments have been made to determine the transition periods between the two types of heart curve. Histological studies of the variations in the secretory appearance of the frog's thyroid have been made by Sklower (1925) and Meisenheimer (1936) and, if the agency effecting the alteration in the relation between heart rate and temperature is the thyroid hormone, it might be expected that seasonal data would provide physiological confirmation of the earlier histological observations. From September 1946, the form of the temperature-pulse rate curve of the isolated heart was determined on samples of frogs collected at monthly intervals throughout the year. In the course of this work it soon became apparent that the curves obtained could not be simply classified into the two types described by Barcroft & Izquierdo (1931), but that other types were also appearing. This rendered interpretation of their seasonal distribution in terms of thyroid activity impossible, as the factors leading to the appearance of the new types were not known. One of these new types of curve was assumed to be due to the uncomplicated action of temperature on the pulse rate, and was selected for further experimental work. Various endocrine preparations were added to the media perfusing such hearts, and it was found possible to reproduce the other types of curve which had been obtained from normal hearts. This paper presents a description of the various types of curve obtained in the seasonal survey, and their possible analysis in the light of these experimental results. Finally their seasonal distribution, particularly with reference to thyroid activity, is considered.

MATERIAL AND METHODS

The frogs used in the seasonal survey were all obtained from a collector in Shropshire, and on arrival in the laboratory were kept in a well-lit and ventilated room where the temperature was approximately atmospheric. In view of the time involved in setting up the heart preparation and the determination of the temperature-pulse rate curve it was not possible to carry out more than one experiment a day. It was desirable to work with a minimum sample number of twelve animals, so that it was inevitable that the later observations in each month were made on frogs which had been in captivity and unfed for periods as long as 14 days. With a view to minimizing this delay the perfusion apparatus was duplicated so that two hearts could be set up side by side and their beat recorded on the same drum. This arrangement was also found to be very convenient in the later experimental work, as it enabled parallel experiments to be made. In general, from the beginning of 1947 onwards two slightly smaller samples were obtained each month, so that the delay in completing the observations rarely exceeded 1 week after capture, and also the total monthly sample number was increased to between fifteen and twenty animals.

The heart was isolated from the frog, after pithing, by ligaturing the anterior venae cavae and tying a cannula into the inferior vena cava. The heart was then removed to a perfusion apparatus essentially similar to that described in detail by Carter (1933). In the present work no aortic cannula was used, so that the heart discharged freely into the beaker in which it was suspended. Adequate aeration of the medium was ensured by bubbling air into the beaker containing the heart, and by using an air-lift system to return the fluid, after perfusion, to the upper reservoir. One litre of Ringer solution was used for perfusing each heart. A venous pressure of 2–3 cm. of water was sufficient to ensure filling of the heart without auricular distension. The venous cannula was made from drawn-out $\frac{3}{4}$ in. tubing, and the upper part of the cannula served as a reservoir in which temperature equilibration of the Ringer with that in the surrounding beaker took place before it entered the heart. In view of the volume of this part of the cannula, and the depth to which it was immersed in the beaker, it is not thought that there was any significant difference in the temperature of the Ringer in contact with the two sides of the heart. The temperature of the Ringer was kept constant at any particular value for 5 min. before the rate of beat was recorded, and no experiments were started until the heart had been isolated and under perfusion for approximately 2 hr. The rate of beat was usually recorded at 2° intervals over the range 7–17° C. This range was adequate to show the difference between the two types of curve described by Barcroft & Izquierdo (1931), and there was no danger of exposing the heart to too high a temperature. In the later work, where the effect of various extracts on the heart curve was under investigation, the temperature range was often extended to 21° C.

The perfusion fluid used was a Ringer solution of the composition given by Carter (1933), and the pH was adjusted to 7·6 by phosphate buffers before the commencement of each set of observations. All the seasonal curves were obtained by perfusing this Ringer after the addition of adrenaline, whereas Carter used unmodified Ringer. The concentration of adrenaline used varied from 1 in 5×10^6

to 1 in 2×10^7, the greater part of the observations being made with a concentration of 1 in 10^7. The heart was perfused with adrenaline-Ringer immediately after isolation, the adrenaline being renewed at the same concentration 20 min. before frequency observations were started. The order of temperature change used in this part of the work was 13, 15, 17, 13, 11, 9 and 7° C., and the experiments were completed in about $1\frac{1}{4}$ hr. from the second adrenaline addition.

TYPES OF TEMPERATURE-PULSE RATE CURVE

When the seasonal determination of temperature-pulse rate curves had been completed it was found that the curves obtained could be classified into five main types (Fig. 1). In order to obtain these curves the mean rate of beat at 2° C. intervals over the temperature range 7–17° C. has been calculated from the rates determined for each heart in the particular group. The data on which Fig. 1 is based are given in Table 1, together with the number of hearts in each class and the mean temperature coefficient (Q_{10}) over the whole temperature range.

Table 1. *Mean rates of beat at different temperatures, and temperature coefficients (Q_{10}), for the five types of temperature-pulse rate curve*

Type	Number of hearts	Mean rate at						Q_{10} (7–17° C.)	Q_{10} (7–9° C.)*
		7° C.	9° C.	11° C.	13° C.	15° C.	17° C.		
A	44	19·7	24·4	29·6	35·4	42·1	49·6	2·52 ±·022	2·18
B	61	19·8	24·5	30·4	36·4	42·8	49·2	2·49 ±·017	2·20
C	34	20·5	26·5	32·6	39·0	45·6	52·1	2·54 ±·023	2·54
D	8	19·7	24·2	31·0	37·8	42·9	47·8	2·43 ±·034	2·16
E	35	20·9	25·5	30·2	34·6	39·6	44·4	2·12 ±·022	2·12

* Value calculated for the line obtained by extrapolation of the 7 and 9° C. rates to 17° C. (see text).

Type *A* which shows an exponential relation between temperature and pulse rate corresponds to the 'winter' heart described by Barcroft & Izquierdo (1931). Similarly, type *C*, where the increase in frequency is linear over the temperature range, corresponds to the 'summer' curve. Type *B* is very similar to type *A*, but has been separated from the latter because the pulse rates lie on two straight lines intersecting at about 10° C., and do not form a smooth curve. There is no significant difference in the observed temperature coefficients for these three types of curve. Type *D* is of very distinctive appearance and was observed only rarely. The pulse rates for the upper and lower temperatures lie on two straight lines of very similar slope, but which would have different origins at 7° C. Type *E* is the same as type *C* in that the pulse rates lie on a straight line, but has been separated from the latter because the hearts in this group show a significantly lower temperature coefficient ($P < 0{\cdot}001$). The seasonal distribution of these various types of curve will be dealt with below (p. 160).

In Fig. 1 the broken lines in curves *A*, *B* and *D* show the projection to 17° C. of the line joining the pulse rates at 7 and 9° C. It is apparent that this line in these types has a very similar slope. Thus the temperature coefficient calculated for this hypothetical line between 7 and 17° C. only varies from 2·16 to 2·20 (last column of Table 1). In the case of type *C*, where the overall relation is linear, the projection of the 7 and 9° C. rates also describes the path of the observed pulse rates at the higher temperatures. This is also the case with type *E*, except that here all the rates observed lie on practically the same line as the theoretical projection obtained from types *A*, *B* and *D*. A similar line (Q_{10} 2·12) is also obtained if the line joining the mean rates observed at 13, 15 and 17° C. for type *D* curves is produced backwards to 7° C. (dotted line in Fig. 1).

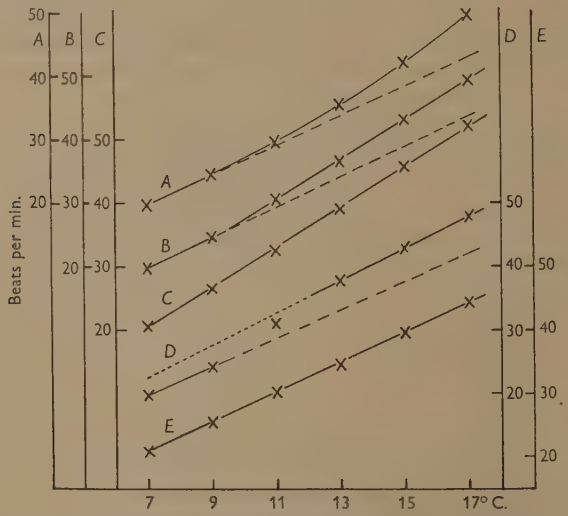

Fig. 1. Various forms of the temperature-pulse rate curve observed during 1946–47. ×———×, mean rates at 2° temperature intervals (from Table 1); — — —, projection of the line joining the rates at 7 and 9° C. to 17° C.; – – – – –, projection of the line joining the rates at 13, 15 and 17° C. to 7° C.

After consideration of these various curves in this manner, it seemed very probable that a linear relation with a Q_{10} value of about 2·15 was a basic component of all of them. This would mean that type *E* where all the rates lay on such a basic line was the least complex relation between temperature and pulse rate. It was therefore decided to investigate the action of various endocrine extracts on hearts of this type in order to identify the factors evoking the appearance of the more complex forms.

EXPERIMENTAL METHODS

There is a high incidence of the type *E* curve during January and the early part of the breeding season (p. 160), and for this reason the experimental work was done at this time of the year. The heart was isolated and set up under perfusion in the manner

previously described. When testing the action of endocrine extracts on the heart the general procedure was as follows. After the isolated heart had been beating under perfusion for approximately 2 hr. the Ringer was removed from the apparatus, and, unless the effect of adrenaline was the specific object of the experiment, adrenaline in a concentration of 1 in 10^7 or 1 in 2×10^7 was added. The Ringer was then re-adjusted to pH 7·6 by adding phosphate buffer (Na_2HPO_4), and perfusion started again. Records of the rate of beat at 2° C. intervals over the temperature range 7–17° C. or 7–21° C. were started 20 min. after the adrenaline addition. The usual practice was to start the observations at 7° C. and raise the temperature progressively to the upper limit. This was done for experimental convenience, as it was found that raising the temperature of the heart vessel could be effected more rapidly than temperature reduction. In general, a full set of observations could be completed within 70 min. from the adrenaline addition.

At the end of the preliminary control experiment the particular endocrine extract under investigation was added to the Ringer in appropriate concentration, and the heart left under perfusion for 1½–2 hr. At the end of this time the Ringer was re-buffered, adrenaline added at the same concentration as before, and another set of observations made. The direction of temperature change, time between observations, and total time taken for the experiment, were, as far as possible, the same in both experiments. In some cases it was necessary to make a third set of observations after further alteration of the medium. The third experiment was carried out in the same way as the second, and, although in such cases the heart had been isolated for approximately 9 hr., the rate and amplitude of beat were usually well maintained. Occasionally hearts were kept under perfusion for 24 hr. after isolation, when a definite change to the hypodynamic state showing reduction in both rate and amplitude was generally apparent (Clark, Eggleton, Eggleton, Gaddie & Stewart, 1938).

EXPERIMENTAL RESULTS

Carter (1933) found that pituitrin, thymus extract, and extracts of non-endocrine organs such as muscle and salivary glands, did not affect the form of the temperature-pulse rate curve. In the present work preliminary experiments were made to investigate the action of acetylcholine, eserine, atropine, pitressin, and oxytocin on the type *E* curve. While some of these agents materially changed the rate of beat of the heart, none of them produced any significant change in the relation between temperature and frequency. Treatment of the heart with an extract of anterior pituitary gland, with thyroxine or with adrenaline was, however, found to lead to alteration in the temperature-pulse rate curve.

(i) *Anterior pituitary extract*

Through the courtesy of Organon Ltd. a supply of a general extract of the anterior pituitary was made available. This extract was prepared by the method described by Young (1941), and will be referred to as Young's Pituitary Extract (Y.P.E.). The addition of 0·5 ml. of Y.P.E. (≡125 mg. of fresh gland) to the litre of Ringer perfusing the heart led to a marked and reproducible alteration of the type *E* temperature-

pulse rate curve. The record of a typical experiment is shown in Fig. 2. The control experiment with adrenaline 1 in 10^7 gave the linear, low Q_{10}, relation characteristic of the *E* type. The second experiment, however, after the addition of 0·5 ml. Y.P.E. per litre shows that, although the rates of beat at 7 and 9° C. were identical in both cases, there has been an increase in rate at the higher temperatures. Not only has there been an increase in rate, but also the rates at 13, 15 and 17° C. lie on a line of similar slope to that found in the first experiment, but which would have a higher origin at 7° C. (broken line in Fig. 2). Thus the type *E* relation has been changed to type *D* (Fig. 1) by the anterior pituitary extract. The observed Q_{10} between 7 and 17° C. for the second experiment is 2·38 compared with 2·15 for the first experiment, while the upper line in the second experiment would have given Q_{10} 2·13 over the whole range. This characteristic change in the temperature-pulse rate curve after the addition of Y.P.E. to the medium was confirmed by many experiments.

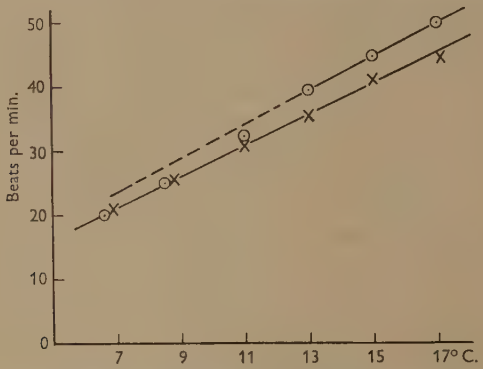

Fig. 2. Action of anterior pituitary extract (Young, 1941) on type *E* heart. Frog no. 305, ♀, 30 April 1948. ×———×, perfused with Ringer containing adrenaline 1 in 10^7; ⊙———⊙, perfused with same solution containing 0·5 ml. Y.P.E. per litre; — — —, projection of line joining 13, 15 and 17° C. rates to 7° C.

An almost identical response was obtained after adding 0·5 ml. of Antoxylin (Oxoid Ltd.) to the Ringer. This preparation is stated to be equivalent to 65 mg. of desiccated anterior pituitary per 0·5 ml. The response with Antoxylin differed slightly from that with Y.P.E. in that the change-over from the lower to the higher linear relation was not as abrupt. In some cases it was only above 17° C. that stabilization of the frequency acceleration on a second straight line was indicated. The basic nature of the response, however, was the same, and minor discrepancies may perhaps be attributed to quantitative differences between the extracts.

Experiments have also been made to test the action of crude extracts of frog anterior pituitary on type *E* hearts. These extracts were made by grinding up eight to ten anterior pituitaries, from freshly killed frogs, in Ringer with a little sand, and leaving them to extract for a few hours at room temperature and pH 8·5. The extract was then added to the perfusing Ringer, the volume of which in view of the dilute nature of the extract, was reduced to 500 ml. in these experiments.

The typical response obtained from frog pituitary extract is shown in Fig. 3*A*. Again it is apparent that the pituitary extract has not altered the rate of beat at 7 and 9° C., but at higher temperatures there is a continuous increase in rate relative to the control experiment up to the temperature limit (20·5° C.), and the observed Q_{10} was increased from 2·22 to 2·40. This was typical of the response obtained with frog extract, and there was never any indication of the acceleration stabilizing on a second straight line of higher origin. In fact, the original type *E* relation has been changed to one of type *B* (Fig. 1). Whether this should be interpreted as a fundamental difference in the properties of the mammalian and frog pituitary extracts is not as yet quite clear. There is a very obvious difference in the concentration of the extracts, as 0·5 ml. Y.P.E. represents 125 mg. of fresh gland, while ten frog anterior

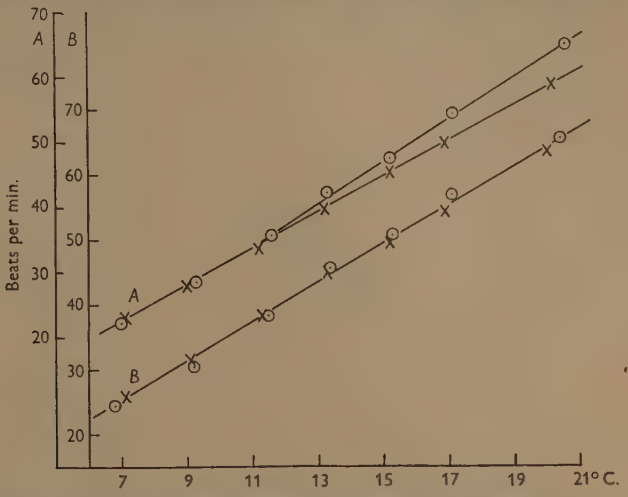

Fig. 3. Action of extract of frog anterior pituitary on type *E* heart, 13 February 1950. *A*, ×———×, heart perfused with Ringer containing adrenaline 1 in 2×10^7; ⊙———⊙, same solution containing extract of ten frog anterior pituitaries per 500 ml.; *B*, ×———× and ⊙———⊙, control heart perfused with Ringer containing adrenaline 1 in 2×10^7.

lobes would weigh only a few milligrams, so that the difference is again most probably a quantitative one. However, despite certain differences in the response, it is quite clear that the change in the temperature-pulse rate curve caused by all these anterior pituitary extracts was effected by an increase in the rate of beat above 9° C. This may lead to a new linear relation after a short transition period, or may be progressive up to the highest temperature observed.

The record of a typical control experiment is shown in Fig. 3*B*. This heart was mounted in the second unit of the perfusion apparatus, and its treatment was identical with that of the heart which behaved as shown in Fig. 3*A*, except that two consecutive sets of observations were made after the addition of adrenaline 1 in 2×10^7 to the Ringer, no pituitary extract being used. In this case it can be seen that the rates

observed in both experiments show no significant departure from the typical type *E* relationship. The experimental addition of anterior pituitary extract has in no case resulted in the production of the 'winter' or type *A* exponential relation. Even after treatment with frog pituitary extract (Fig. 3) the temperature-pulse rate curve consists of two straight lines intersecting at about 11° C. Again this is perhaps a quantitative effect. All the extracts used in these experiments would be expected to contain a higher concentration of the active principle than that occurring naturally in the frog. With the most effective extract used (Y.P.E.) the full effect of the pituitary principle is produced between 9 and 13° C. (Fig. 2), but with a weaker preparation the action has not developed fully even at 20° C. It, therefore, seems very probable that in the presence of even lower concentrations of the pituitary hormone, the development of the synergistic action at intermediate temperatures might be depressed, while still being quite marked at the higher temperatures. This would lead to the appearance of an exponential relation, instead of the type shown in Fig. 3.

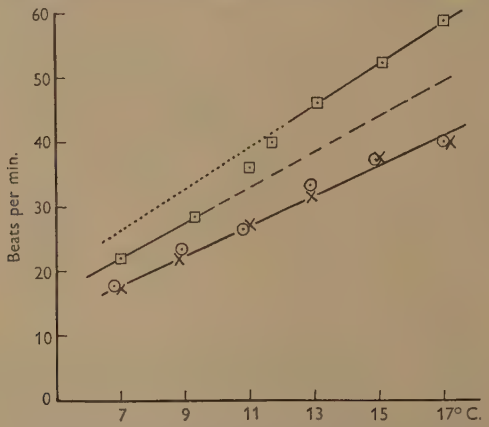

Fig. 4. Synergistic action of anterior pituitary extract and adrenaline. Frog no. 240, ♀, 21 January 1948. ×———×, perfused with unmodified Ringer; ⊙———⊙, Ringer containing 0·2 ml./l. of Y.P.E.; ⊡———⊡, same solution containing adrenaline 1 in 10^7; – – – – –, projection of line joining rates at 13, 15 and 17° C. to 7° C.

In all the experiments with anterior pituitary extract so far described adrenaline had been added to the perfusate. It was obviously important to ascertain whether adrenaline itself had any influence on the pituitary induced response. To investigate this point a series of triple experiments were made, the results of a typical one being shown in Fig. 4.

The first experiment was made with the heart beating in unmodified Ringer, and the observed rates fit the normal type *E* line quite well. The second experiment was made after the heart had been beating for a further 2 hr. after the addition of 0·2 ml. Y.P.E. to the Ringer. It can be seen that there is no indication of the typical pituitary response described previously. Before taking the third set of readings adrenaline (1 in 10^7) was added to the perfusate. It is now quite clear that in addition to the

expected chronotropic action of adrenaline shown at the lower temperatures, there is also the typical anterior pituitary induced effect at the higher temperatures (cf. Fig. 2). Experiments such as this plainly indicate that the action of anterior pituitary extract on the temperature-pulse rate curve is only produced in the presence of adrenaline. This phenomenon can be most readily explained by assuming that there is a synergistic action between adrenaline and an anterior pituitary factor at the higher temperatures. This synergism results in the positive chronotropic action of adrenaline being facilitated so that there is a relatively greater increase in rate at the higher temperatures, and the observed temperature coefficient between 7° and 17° C. is increased. The above experiments also enable the temperature threshold, above which this synergistic action appears, to be defined. In all cases the extra increase in frequency is absent at 9° C., while it is plainly manifest at 11° C. Thus the threshold lies in the region of 10° C. When mammalian pituitary extract was used the adrenaline facilitation was apparently complete at 13° C., above which temperature the normal acceleration was again observed (Figs. 2 and 4). The frog extract did not lead to such an abrupt facilitation of adrenergic action, and the extra increase in frequency continued over the experimental temperature range. The extent of the frequency increase caused by this anterior pituitary-adrenaline synergism may be quite considerable. For example, in the experiment shown in Fig. 4 the continuation of the line joining the rates observed at 7 and 9° C. in the third experiment (broken line in Fig. 4) shows that a frequency of 38·5 beats per min. would have been obtained at 13° C. if the action of adrenaline had been the same at all temperatures. This would represent an increase of 7 beats per min. above the mean rate at this temperature for the first two experiments. The observed rate at 13° C. was actually 45·5 per min., so that the frequency response to adrenaline has been increased two-fold by the presence of the anterior pituitary principle.

A facilitation of the action of adrenaline by a secretion of the pituitary, similar to that postulated above, was suggested by Kepinov (1938, 1949). Kepinov found that adrenaline had no glycogenolytic action on the perfused liver of the hypophysectomized frog, but the typical action of adrenaline appeared if an extract of normal frog liver or pituitary were added to the perfusion medium. The pituitary extract alone had no glycogenolytic action on the frog liver. Kepinov therefore attributed the marked glycogenolytic action of adrenaline on the frog liver to synergism between adrenaline and a substance of anterior pituitary origin. A reduction of the glycogenolytic action of adrenaline in various hypophysectomized animals, including the frog, was also found by Bodo, Bloch & Gross (1942) and by Fluch, Greiner & Loewi (1935). Cohen (1947) showed that previous hypophysectomy inhibited the decrease in anaerobic glycolysis of rat diaphragm produced by adrenaline injection, and he suggested that a pituitary influence on the action of adrenaline might possibly be involved.

Search of the literature has not revealed any previous work which suggests that the intensity of this anterior pituitary-adrenaline synergism is affected by temperature. All the experiments on the perfusion of the isolated frog's liver mentioned above appear to have been made at room temperature, so that the inhibition of pituitary

action at temperatures below 10° C. found in the present work would not be observed. There does not, at present, seem to be any reason why the inhibitory action of low temperatures might not also be a feature of the facilitation of the glycogenolytic action of adrenaline by an anterior pituitary hormone.

Confirmation of the hypothesis that the most commonly observed relation between the frequency of the isolated frog's heart and temperature is attributable in part to a secretion of the anterior pituitary gland, should be forthcoming from experiments on hearts isolated from hypophysectomized frogs. Such experiments have been made and rather complex results obtained. Removal of the anterior pituitary was effected by Hogben's (1923) method, and the temperature-pulse rate curve determined at varying post-operative intervals. Oriás (1934) found that about 2 weeks after hypophysectomy a marked reduction in pulse rate occurred in *Bufo arenarum* and

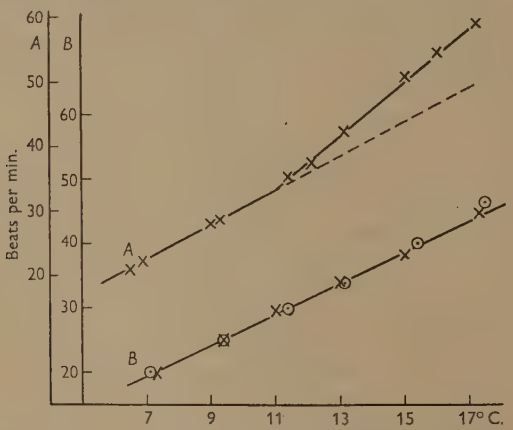

Fig. 5. Effect of hypophysectomy on the temperature-pulse rate curve. *A*, control heart from frog kept in captivity and unfed for 5 weeks. Beating in Ringer containing adrenaline 1 in 2×10^7 (28 February 1950); *B*, heart from frog hypophysectomized (anterior lobe only) 5 weeks previously; ×———×, beating in Ringer containing adrenaline 1 in 2×10^7; ⊙———⊙, same solution containing extract of ten frog anterior pituitaries (3 March 1950).

this has been confirmed in the present work for *Rana temporaria*. Frogs which were hypophysectomized in September 1948 showed a very uniform, low pulse rate when the heart was isolated from 15 to 40 days after operation. The mean frequency at 7° C. for six hearts perfused with Ringer containing adrenaline (1 in 2×10^7) was $14{\cdot}1 \pm 0{\cdot}13$ beats per min. These hearts showed a linear relation between temperature and pulse rate up to 15° C., but a slight extra acceleration occurred at higher temperatures. Another series of similar experiments was started in January 1950, when it was found that up to 5 weeks after operation no bradycardia had developed. A typical experiment made with one of these hearts is shown in Fig. 5*B*. The temperature-pulse rate curve is obviously linear over the whole range (Q_{10} 2·22). Curve *A* in this figure is the record of a parallel experiment on a heart isolated from an unoperated frog which had been in captivity for a similar length of time and

under the same conditions as the operated animals. In those cases, where hypophysectomy was uncomplicated by bradycardia, the appearance of the type *E* heart, which is to be expected on the basis of the above theory, has in fact occurred.

The action of anterior pituitary extract, in the presence of adrenaline, on hearts from hypophysectomized animals was also investigated in some experiments, but in no case was any effect obtained (Fig. 5). This failure of pituitary extract to produce its usual action may perhaps be due to the decreased activity of other endocrine glands as a consequence of hypophysectomy.

Three frogs from the group hypophysectomized at the end of January were kept until the end of March before examination, that is from 7 to 8 weeks after operation. These hearts all exhibited the typical bradycardia found in September 1948, and also the relation between temperature and pulse rate was linear over the range 7–17° C. It may perhaps be of some significance that bradycardia did not develop during the January-February period when a high incidence of type *E* hearts is normally found. At present it certainly seems that the effects of hypophysectomy on the pulse rate are complex, and may be subject to seasonal influences. It is hoped that further work on this problem will be possible in the near future.

(ii) *Thyroxine*

Several experiments were made to test the action of thyroxine alone on hearts showing the type *E* temperature-pulse rate relation. At the end of the control experiment with adrenaline, thyroxine-sodium (B.D.H.) was dissolved in the perfusate at a final concentration of 1 in 10^6. It was found that thyroxine alone did not lead to any significant change in the type *E* curve. The record of a typical experiment is shown in Fig. 6, where the pulse rates after thyroxine addition do not differ materially from those observed in the control experiment.

The action of thyroxine after previous treatment of the heart with anterior pituitary extract was also investigated. Fig. 7 is the record of a triple experiment designed for this purpose. With adrenaline only added to the Ringer the temperature-pulse rate relation is typical of type *E*, and the addition of 0·5 ml. per litre of anterior pituitary extract (A.P.E.) causes a change-over to type *D* (Fig. 1). In the third experiment, which was made after the heart had been in the same solution plus thyroxine-sodium (1 in 10^7) for a further $1\frac{1}{2}$ hr., another change is apparent. All the observed rates over the experimental temperature range now fall on a straight line of steeper slope than that for the first experiment, that is a type *C* (Fig. 1) curve has been produced. Examination of Fig. 7 shows that this linear relation has resulted from the smoothing out of the sharp discontinuity between the two similar lines obtained with adrenaline and pituitary extract. In addition, in the presence of thyroxine the increased acceleration is maintained at the higher temperatures, so that there is no indication of stabilization on a second line of low-temperature coefficient.

The effect of thyroxine shown in Fig. 7 corresponds to the conversion of the normal winter heart curve into the summer type described by Carter (1933). Carter states that 'the rate of increase of beat begins to fall off towards the upper limit of

the range of temperature at a lower point' in the winter heart, so that 'the winter curve is often S-shaped within the temperature range and the summer curve is not'. Re-examination of some of Carter's published data indicates that this change in the

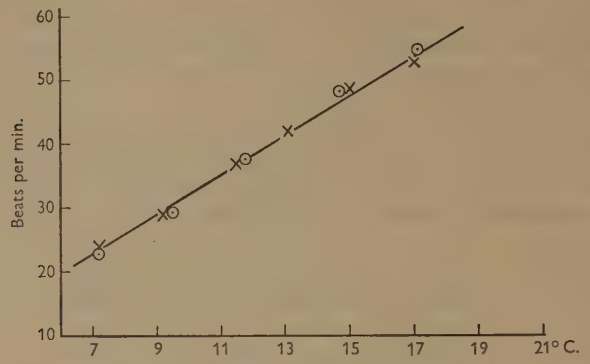

Fig. 6. Action of thyroxine on the type *E* heart, 14 February 1950. ×———×, beating in Ringer containing adrenaline 1 in 10^7; ⊙———⊙, same solution containing thyroxine-sodium (B.D.H.) 1 in 10^8.

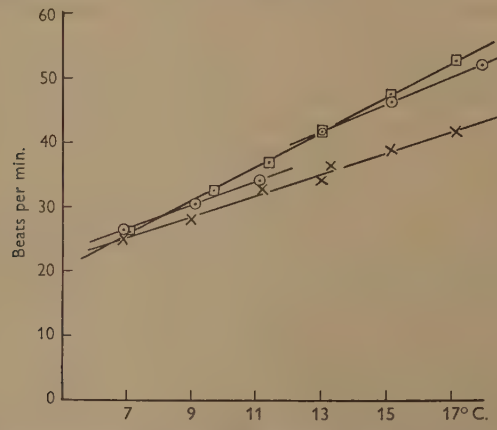

Fig. 7. Action of anterior pituitary extract and thyroxine on the type *E* heart. Frog no. 254, ♀, 4 February 1948. ×———×, beating in Ringer containing adrenaline 1 in 2×10^7; ⊙———⊙, same solution containing 0·5 ml. of Y.P.E. per litre; ⊡———⊡, same solution as in second experiment, plus thyroxine-sodium 1 in 10^7.

shape of the curve at the higher temperatures in the case of the winter heart is probably due to the acceleration of pulse-rate stabilizing on a straight line with a relatively low-temperature coefficient. In the normal winter heart, however, such stabilization only appears at about 20° C., so that this interpretation is only possible by analogy with the experimental results after anterior pituitary treatment. The present work, therefore, clearly confirms Carter's finding that addition of thyroxine to the medium

perfusing the frog heart can change the exponential temperature-pulse rate curve to a linear form. Further, it is only necessary to expose the heart to such a medium for 1½–2 hr. to obtain a positive result.

The effect of thyroxine on the temperature-pulse rate curve of 'winter' frogs when injected into the animal has also been investigated. These experiments were made between September and December on frogs which were kept at a temperature of 4–6° C. Some of these animals were injected with 1 ml. of a 1 in $1{\cdot}7 \times 10^6$ solution

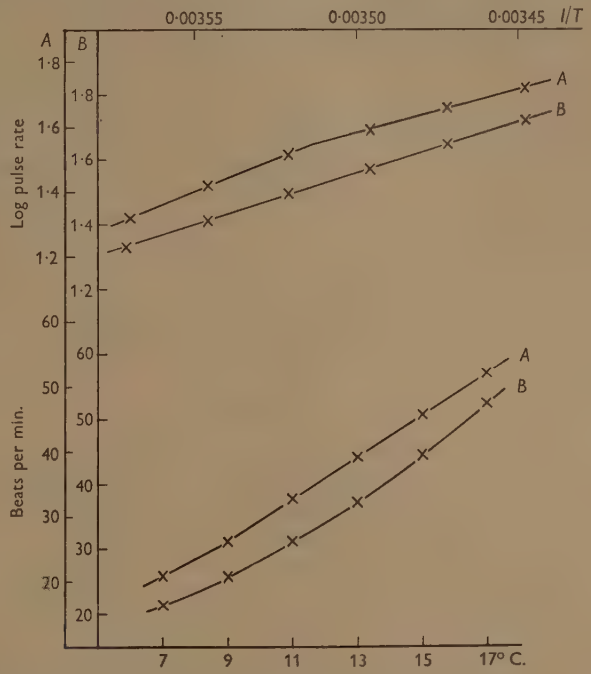

Fig. 8. Effect of previous thyroxine injections on the temperature-pulse curves of hearts isolated from frogs kept in the cold. *A*, mean rates for four hearts from frogs injected with 1 ml. of a 1 in $1{\cdot}7 \times 10^6$ solution of thyroxine-sodium daily; *B*, mean rates for four hearts from control frogs. Upper part of the figure shows the same rates plotted on a logarithmic scale.

of thyroxine-sodium daily, while others were retained as controls. The period of preliminary exposure to cold and thyroxine treatment varied from 6 to 13 days. The mean curves for hearts isolated from four control (*B*) and four thyroxine-treated (*A*) frogs are shown in Fig. 8. It is apparent that the control curve is of an exponential type, and plotting the logarithm of the rate against the reciprocal of the Absolute temperature, as shown in the upper part of Fig. 8, gives a straight line. The frequency at the lowest temperature is not changed by thyroxine treatment, nor is the ultimate rate attained at 17° C. The difference between the two curves lies in the fact that

the increase in rate is greater at the intermediate temperatures after thyroxine treatment, so that the curve approaches a linear form, and logarithmic plotting now shows two intersecting straight lines. The effect of thyroxine injected into frogs kept in the cold is therefore essentially similar to that produced by the addition of thyroxine to the medium perfusing the isolated heart.

It is well known that the thyroid hormone increases sensitivity to adrenaline (Best & Taylor, 1950; Bacq, 1936; Ring, 1942). Schaeffer & Thibault (1945) have shown that this synergistic action of thyroxine with adrenaline is independent of its normal metabolic action. They found that injection of adrenaline into white rats 10 hr. after a previous thyroxine injection led to a 45 % increase in metabolic rate, although the effect of the thyroxine injection itself was either very weak or not detectable after such an interval. Similarly, the facilitation of adrenergic action persisted for a considerable time after the decay of the metabolic action of the injected thyroxine. In both Carter's (1933) and my own experiments it has been found that the action of thyroxine on the temperature-pulse rate curve is apparent within $1\frac{1}{2}$ hr. of its addition to the perfusing medium. In view of this short latent period it seems very probable that thyroxine alters the form of the curve by virtue of its synergistic action with adrenaline. The present experiments have also shown, however, that the change in form of the temperature-pulse rate curve caused by this interaction between thyroxine and adrenaline is conditional on the presence of an anterior pituitary principle (Figs. 6, 7).

(iii) *Adrenaline*

There is some conflict in the literature regarding the effect of adrenaline on the temperature coefficient of the isolated frog's heart. Carter (1933) found a general increase in the rate of beat after adrenaline addition, but no change in the shape of the temperature-pulse rate curve. Gellhorn (1924) found that the temperature coefficient of strips of frog's heart muscle was increased after adrenaline. It has been shown in the preceding sections that both the anterior pituitary factor and thyroxine appear to change the form of the temperature-pulse rate curve by virtue of a synergistic action with adrenaline. These experiments do not, however, give any indication of the action of adrenaline itself on curves other than type *E*. In the autumn and early winter of 1947 a set of observations was made with the heart beating in unmodified Ringer, before making the usual records after the addition of adrenaline. A total of thirty-three experiments with and without adrenaline was made in this series. Examination of the records showed that adrenaline could either increase, decrease, or have no effect on the temperature coefficient. The records were subdivided into three groups according to the change in Q_{10}, and the mean rates at each temperature calculated for each group. The means of the observed rates, with and without adrenaline, at 7 and 17° C., together with the mean value for Q_{10} over the range 7–17° C. are given in Table 2. As the linear projection of the rates at 7 and 9° C. was found to be a basic feature of the temperature-pulse rate curve in the presence of adrenaline (Fig. 1), the value of Q_{10} for this projection has been included in the last column of Table 2. The data are shown graphically in Fig. 9,

where the broken lines indicate the projection of the line joining the frequencies at 7 and 9° C.

Table 2. *The effect of adrenaline* (1 *in* 10^7) *on the rate of beat, and temperature coefficient* (Q_{10}), *of the isolated frog's heart*

Group	No. of hearts	Adrenaline	Rate at 7° C.	Rate at 17° C.	Increase in rate at 7° C. with adrenaline	Q_{10} (7–17° C.)	Q_{10} (7–9° C.)*
I	8	−	17·4	43·1		2·48	2·25
		+	19·8	48·1	2·4 ± 0·429	2·48	2·23
II	17	−	17·0	39·8		2·34	2·18
		+	18·7	48·1	1·7 ± 0·185	2·57	2·20
III	8	−	17·5	44·8		2·51	2·44
		+	21·6	48·7	4·1 ± 0·60	2·25	2·13

* Value calculated for the line obtained by extrapolation of the 7 and 9° C. rates to 17° C. (see text).

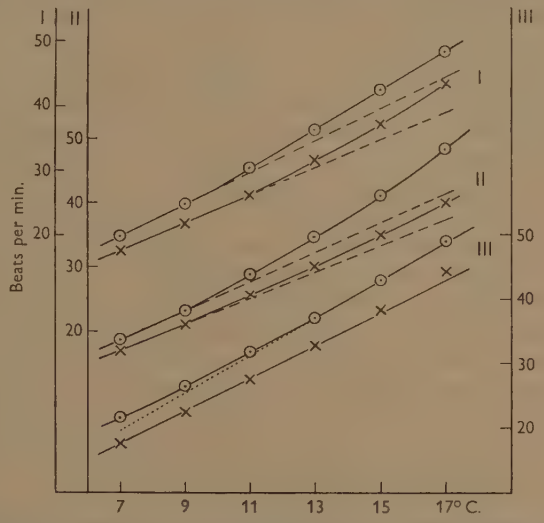

Fig. 9. The effect of adrenaline on the temperature-pulse rate curve. Data from Table 2. ×———×, mean rates for hearts beating in unmodified Ringer; ⊙———⊙, mean rates in Ringer containing adrenaline 1 in 10^7; — — —, projection of the line joining the rates at 7 and 9° C. to 17° C.; ·····, suggested course of true curve for group III (see text).

It is evident that the hearts in group III are of a different type from those in the two other groups. The observed rates without adrenaline in group III nearly all lie on the straight line of Q_{10} 2·44 which has been drawn in Fig. 9. This indicates that these hearts probably correspond to the usual type *C* (Fig. 1). The mean increase of rate after addition of adrenaline shown by these hearts is practically twice that found in groups I and II (Table 2). Statistical tests of significance applied to the increases in frequency with adrenaline at 7° C. in groups II and III show that *P* is less than

0·001, and in groups I and III that P is 0·04, so that the greater increase in group III is probably significantly different from that found in the other groups. The means of the observed rates with adrenaline for the group III hearts lie on a slight curve with a Q_{10} value of 2·25. Close inspection of Fig. 9 shows that the rates at 13, 15 and 17° C. in the presence of adrenaline lie on a straight line, and if this line is produced backwards (dotted line in Fig. 9) its origin at 7° C. would give a pulse-rate of 19·5 per min. Such a rate is 2 beats per min. faster than the mean rate without adrenaline at the same temperature. It is therefore suggested that the decrease in temperature coefficient caused by the addition of adrenaline to this type of heart is due to an abnormally high frequency response at the lower temperatures. Owing to the upward temperature gradient used in these experiments the rate at 7° C. was the first observation to be made after the adrenaline was added, and the abnormally high value obtained is very probably a direct result of this procedure. It would seem that this phenomenon only occurred with type C hearts, that is those isolated from frogs with active thyroid glands. Experiments with type *E* hearts have shown that thyroxine may act synergistically with adrenaline even at low temperatures (Fig. 7). Further, Bacq (1936) has shown that thyroxine inhibits, *in vitro*, the destruction of adrenaline in solution. He attributed the greater sensitivity to adrenaline found in hyperthyroid subjects, and in animals injected with thyroxine, to a more rapid and longer lasting reaction of the tissues in the presence of a greater amount of the thyroid hormone. It is quite possible, then, that adrenaline added to hearts containing the thyroid hormone leads to a transient increase in pulse-rate which outlasts the 20 min. stabilization period observed in this work. The decrease in temperature coefficient after adrenaline observed in these hearts is, therefore, attributed to an experimental artefact caused by the technique employed. It is suggested that the true rates after the addition of adrenaline should lie along the dotted line drawn in Fig. 9. If this is accepted, then there is no real change in temperature coefficient caused by adrenaline, and the pulse-rate change is linear over the whole range.

This abnormal response at the lower temperatures did not occur in the normal seasonal observations because the heart had been perfused with adrenaline-Ringer for 1½–2 hr. after isolation, and the observations were not started at the lower end of the temperature range. With the procedure used throughout the seasonal work normal type *C* or 'summer' curves were observed in the presence of adrenaline.

Comparison of the curves obtained with and without adrenaline for groups I and II shows that the increased temperature coefficient after the addition of adrenaline in group II is due to a greater acceleration in rate over the upper part of the temperature range. The difference in reaction toward adrenaline found in these two groups is capable of explanation if it is assumed that the isolated heart beating in unmodified Ringer is subject to the influence of an endogenous adrenergic substance. There is considerable evidence supporting the view that adrenergic compounds are present at sympathetic nerve endings (Bacq, 1949). Raeb & Maes (1947) found a marked diminution in the concentration of an epinephrine-like substance in the cat's heart 7 days after total sympathectomy and adrenal inactivation, but even then there remained a certain amount of residual material which seemed to be chemically identical with

epinephrine, though they suggested that it might differ physiologically. Hoffman, Hoffman, Middleton & Talesnik (1945) found that injection of acetylcholine or nicotine into the isolated, atropinized hearts of dogs, cats, rabbits and guinea-pigs, led to the release of an epinephrine-like substance into the perfusion medium. On the other hand, they also found that the addition of acetylcholine or nicotine to the atropinized perfusion fluid had no epinephrine-like action on the isolated heart of *Rana pipiens*. McDowall (1946), however, showed that acetylcholine under certain circumstances could stimulate the isolated frog's heart. This action was enhanced by treatment of the heart with eserine after atropine, but was abolished by ergotoxine. It seems, therefore, quite probable that the frog's heart may show symptoms of an adrenergic influence for several hours after isolation. If this indeed be the case, then it is suggested that curves of the group I type are obtained because the heart beating in Ringer contains enough of an active adrenergic principle for the synergistic action of the anterior pituitary factor to be fully developed. Therefore addition of adrenaline to the perfusate only leads to a proportionate increase in frequency at all temperatures, with no change in the observed temperature coefficient. In the case of the group II hearts, however, it may be that the heart beating in unmodified Ringer contains less of the adrenergic material so that the full potentiality of anterior pituitary action at higher temperatures cannot be developed. In this case it would be expected that addition of adrenaline would lead to a disproportionate increase in frequency at the higher temperatures, and hence to an increase in the observed temperature coefficient. It must also not be forgotten that variations in the amount of the anterior pituitary factor contained in the heart might possibly contribute to a difference in behaviour when extra adrenaline is added to the medium.

The hypothesis that the isolated heart may be subject to a persistent adrenergic influence is also supported by several incidental experimental observations. When the heart was perfused with Ringer for periods of 24 hr. it frequently went into the hypodynamic state, characterized by a 50 % reduction in frequency and considerable decrease in amplitude. Hearts which, when first isolated, gave temperature-pulse rate curves of type *A* or *B* changed to a linear form over the whole temperature range when they became hypodynamic.

Secondly, on a few occasions when the temperature-pulse rate curve was being determined for hearts beating in unmodified Ringer, results were obtained which might be regarded as indicative of the disappearance of an adrenergic factor. Such a case is illustrated in Fig. 10. The frequency observations at the lower temperatures in the first experiment, which was started $1\frac{3}{4}$ hr. after isolation, show a high rate of beat. Above 12° C. there was an obvious decrease in acceleration with further rise in temperature. After the 20° C. rate was recorded the temperature was reduced by stages to 7° C. again. The rates observed on this falling temperature gradient formed a straight line (Q_{10} 2·22) which passed through the 20° C. observation. The observed rates at 7° C. differed by 4·5 beats per min. Adrenaline (1 in 10^7) was now added to the Ringer and it can be seen that the reduction in pulse-rate was more than overcome. The acceleration over the lower part of the range as the temperature was raised, was identical with that found for the falling temperature gradient in the

absence of adrenaline. In the presence of adrenaline, however, instead of the linear form being maintained, the increase in frequency became much greater at the higher temperatures and a typical type *A* curve was obtained. It seems possible that this effect at the higher temperatures was not observed in the first experiment because of the exhaustion of the endogenous adrenergic material.

Gellhorn (1924) classified frogs' hearts into two types according to the magnitude of the temperature coefficient of the heart muscle strips. Both types showed an exponential relation between temperature and rate of beat, but one gave temperature coefficients varying from 2·0 to 2·4, while the other gave values up to 3·1. The low temperature coefficient of the first type was associated with a significant fall in amplitude at higher temperatures, while in the second type the change in amplitude

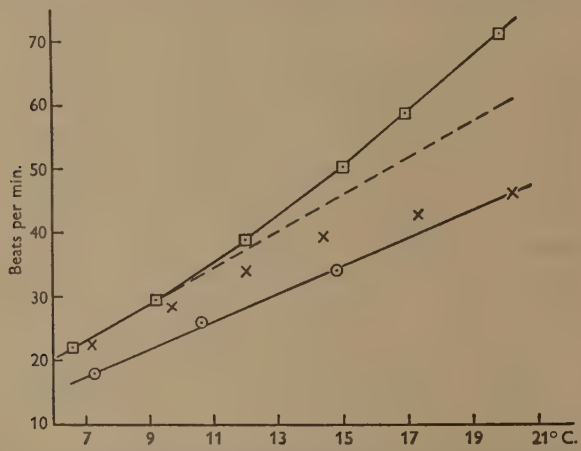

Fig. 10. Disappearance of a sympathetico-mimetic effect in unmodified Ringer. ×———×, heart beating in unmodified Ringer 1¾ hr. after isolation, with temperature increasing; ⊙———⊙, in same solution but temperature falling; ⊡———⊡, in Ringer containing adrenaline 1 in 10^7; — — —, projection of line joining 7 and 9° C. rates to 17° C.

was small. In view of the positive chronotropic and inotropic action of adrenaline, it is possible that Gellhorn was dealing with hearts subjected to intrinsic adrenergic influences of varying intensities, as was suggested for the groups I and II hearts shown in Fig. 9. Gellhorn, however, found that both types of heart gave an increased temperature coefficient with adrenaline (1 in 10^5), so that his results are not strictly parallel to mine. In my experiments it was very frequently observed, in both normal hearts and in those treated with anterior pituitary extract, that the disproportionate increase in frequency at the higher temperatures was accompanied by a distinct increase in the height of the mechanical record. Barlow & Sollmann (1926) also found that not only the actual but even the percentage rate-response to adrenaline (1 in 10^7) of the isolated heart of *Rana pipiens* increased with warming, up to a fairly high temperature (30° C.). Their data also indicate that the frequency-

response to adrenaline is almost constant below 8° C., the disproportionate increase in rate being confined to the higher temperatures.

With a view to obtaining more direct evidence for the existence of a persistent adrenergic action, experiments have been made to test the action of adrenergic blocking agents on the form of the temperature-pulse rate curve of the heart beating in unmodified Ringer. As yet, only a few preliminary experiments have been made with ergotamine tartrate (1 in 10^5). The results have been equivocal, but there is certainly an indication that curves of type *B* may be changed to type *E* by such treatment. It is hoped to extend these experiments in the near future.

If the theory that the isolated heart may still be under the influence of sympathetico-mimetic substances be accepted, then it will also explain the fact that Barcroft & Izquierdo (1931) obtained their summer and winter type curves without adding adrenaline to the medium. Similarly, Carter (1933) demonstrated the action of thyroxine on the winter type of curve when using adrenaline-free Ringer. If there is sufficient adrenergic material in the recently isolated heart for the synergistic action of the anterior pituitary and thyroid hormones to be effective, then there is no conflict between the previous observations and those reported in this paper.

Lichtneckert & Straub (1949) have shown that the frog's heart rendered hypodynamic by treatment with quinine resumes normal activity if either adenosine-triphosphate (ATP) or adrenaline is added to the medium. They suggested that ATP is either identical with, or a precursor of, a substance necessary for the initiation of muscle contraction, and that adrenaline catalytically influences the formation of this substance. In view of this suggestion, investigation of the action of ATP on the form of the temperature-pulse rate curve might prove to be of considerable interest.

(iv) *Conclusions*

As a result of these experiments involving the addition of various endocrine preparations to type *E* hearts the following general conclusions may be drawn.

(*a*) The uncomplicated action of temperature on the frequency of the isolated heart shows a linear relation over the temperature range 7–17° C. The mean temperature coefficient (Q_{10}) for this line is 2·10 (type *E* curve).

(*b*) The exponential relation between frequency and temperature described by Barcroft & Izquierdo (1931) is the result of a synergistic action between an anterior pituitary hormone and adrenaline, which is not operative below 10° C. The linear, summer, temperature-pulse rate curve is a result of the additional facilitation of adrenaline action by thyroxine, which is not limited by a temperature threshold. The presence of the anterior pituitary factor increases the value of the observed temperature coefficient (Q_{10}) to about 2·50.

(*c*) The fact that the addition of adrenaline to the perfusate may either have no effect on, or may increase the temperature coefficient has been attributed to the persistence of active adrenergic material in the isolated heart. If there is an inadequate concentration of this material in the heart then the addition of adrenaline leads to an increase in the temperature coefficient.

SEASONAL DISTRIBUTION OF THE VARIOUS TYPES OF TEMPERATURE-PULSE RATE CURVE

The percentage occurrence of the various types of temperature-pulse rate curve found in the monthly samples, together with the numbers of hearts in each sample, is given in Table 3. While none of the samples yielded curves of a single type, it can

Table 3. *Percentage occurrence of the various types of temperature-pulse rate curve in monthly samples of frogs* (1946–7) *excluding April*

Month	Total no. of hearts	Percentage of type				
		A	*B*	*C*	*D*	*E*
January	15	0	26	7	6	60
February	19	32	26	11	10	21
March	20	5	35	45	0	15
May	19	0	63	21	0	16
June	17	6	35	47	0	12
July	17	6	35	47	0	12
August	17	29	35	24	0	12
September	24	34	29	16	0	21
October	10	30	20	20	20	10
November	8	63	25	0	0	12
December	16	25	38	25	0	12

be seen that certain types predominate at different seasons. Perhaps the clearest example of this is provided by type *E*. For the greater part of the year the incidence of this type was fairly constant at about 15 % of each sample, but in January it increased to 60 %. When experimental work was being done on type *E* hearts in 1949 and 1950 it was found to be the commonly occurring form in February also, although the February sample in 1947 only showed 21 % of this type (Table 3). In 1947 climatic conditions were unusually severe at this time and may have caused modification of the normal seasonal pattern. It has been suggested above that the type *E* curve is produced when a factor of anterior pituitary origin is absent, so that there is apparently a period immediately prior to, and extending into the early part of, the spawning season when this hormone is not being produced in the majority of animals. No attempt has so far been made to identify this principle with any of the recognized hormones of the anterior pituitary gland.

Type *A* hearts were relatively more abundant in the winter months, while type *C* occurred most frequently in the summer. This confirms the findings of Barcroft & Izquierdo (1931), for they called these types 'winter' and 'summer' temperature pulse-rate curves respectively. Type *B* hearts were found in all samples throughout the year. The normal incidence was between 20 and 35 % of the total sample, but a maximum occurrence of 60 % was found in May. The analysis of this type of curve in terms of endocrine activity is rather ambiguous. It is really intermediate in form between types *A* and *C*, and it is possible to envisage its production either by an increased output of the pituitary principle (cf. Fig. 3) or perhaps by moderate thyroid activity. Type *D*, which by analogy with the experimental results is the

result of greater production of the pituitary factor, was only observed six times during the year, and was confined to the winter samples.

Bearing in mind the experimental analysis of these various curves discussed earlier, it is possible to follow the seasonal changes in thyroid activity. The only type of curve which can definitely be attributed to the presence of the thyroid hormone is type *C*. There is a certain amount of doubt about type *E* curves, for, if the pituitary principle is lacking it seems that thyroxine is unable to produce its typical action. It is, therefore, possible that such a relation could be obtained from frogs with active thyroid glands. However, many hearts of this type have subsequently been used for experiments with anterior pituitary extracts, and they have always changed over to types *D* or *B*, but never to type *C* as might be expected if the thyroid hormone were already present. For this reason the type *E* hearts have been

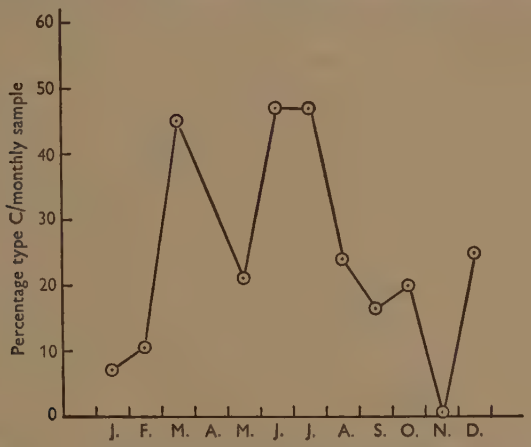

Fig. 11. Percentage occurrence of type *C* temperature-pulse rate curves in monthly samples of frogs (1946–7).

regarded as indicative of thyroid inactivity. In Fig. 11 the percentage of frogs in each sample which gave a type *C* temperature-pulse curve has been plotted for each month. The number of frogs with active thyroids varied from zero in November to about 50 % in June and July. Two well-defined peaks of activity are revealed, in March and in mid-summer, which are separated by a marked decrease in activity in May. From August to November there is a steady fall in thyroid activity, which, with the exception of the December sample, remains relatively low throughout the winter.

Sklower (1925) followed the seasonal changes in thyroid activity in the frog by means of a histological method. He found a low level of activity throughout the winter, followed by a sharp increase in March. The level was high throughout the summer but began to decrease at the end of August, reaching the winter level in October.

Meisenheimer (1936), after more detailed seasonal histological study, summarizes

the changes in thyroid activity as follows. From November to February thyroid function is at a low level, but at the end of February or the beginning of March the activity of the gland rises rapidly and remains high until the end of March or the beginning of April. In April and May there is a pronounced reduction in activity, which may reach the winter level. From June to September there is another very active period, but about the middle of September there begins a phase of decreasing activity which extends into October. This leads to the low, winter level of activity. It is evident that Meisenheimer's description of the thyroid cycle closely parallels that shown in Fig. 11. The fact that even at the summer maximum only 50 % of the samples showed thyroid activity may perhaps be regarded as remarkable. Meisenheimer (1936), however, also stresses the heterogeneous nature of her samples, and states that very different thyroid conditions may be found in frogs caught in the same place on the same day, or even in the two members of a copulating pair. She also showed that the high level of thyroid activity in the mid-summer period is interrupted periodically by the occurrence of moulting. No records of skin condition were made in the present survey and the possible inclusion of moulting animals in the samples would reduce the number showing thyroid activity. The appearance of a slight wave of thyroid activity in December is unexpected, but Meisenheimer also found some glands at this time which in many respects appeared active, although, on the ground of the small number of vacuoles in the colloid, she regarded them as being inactive. It is possible, as Meisenheimer suggests, that mild weather at this time of year may lead to a certain amount of thyroid activity. Meisenheimer further observed that there was no correlation between the high thyroid activity in March and the spawning process. This has been confirmed in the present work, as in the early part of the breeding season hearts isolated from frogs in amplexus usually gave temperature-pulse rate curves of type *E*, which showed no indication of thyroid activity when treated with anterior pituitary extract.

The clear indication of a regression of thyroid activity in April and May found by both histological and physiological methods is of interest in connexion with other work. Morton & Rosen (1949) investigated the seasonal changes in the carotenoid content of frogs. They noted that although frogs feed soon after spawning, carotenoid storage does not occur until the end of May. Cama & Goodwin (1949) found that the thyroid played a part in the metabolism of Vitamin A. Administration of desiccated thyroid stimulated the absorption of B-carotene from the intestinal tract of rats. The failure of carotene storage in frogs in April and May might, therefore, be due to the low level of thyroid activity at this time. Smith (1950) has also shown that over this same period the blood-sugar level is low and there is no appreciable storage of glycogen in the liver, or of fat in the fat-bodies.

Lichtneckert & Straub (1949) found that the action of ATP on the hypodynamic frog's heart was completely absent in the winter season, while there were transition periods in September-November and in March-April when incomplete recoveries were obtained. Again this is a type of seasonal distribution which follows the general lines of the thyroid cycle, and suggests that the possible participation of thyroxine in the action of ATP on heart muscle might merit investigation.

SUMMARY

1. The form of the temperature-pulse rate curve of the isolated frog's heart, when perfused with Ringer solution containing adrenaline, has been determined over the range 7–17° C. for monthly samples of frogs over a whole year. Five different types of curve were obtained during this seasonal survey, namely types *A*, *B*, *C*, *D* and *E*. Of these types, *A* and *C* respectively correspond to the winter and summer temperature-pulse rate curves described by Barcroft & Izquierdo (1931), while the remaining three have not previously been described. Type *E*, which is a linear relation with a mean temperature coefficient (Q_{10}) of 2·12, has been interpreted as being the least complex form, in which the action of temperature on the pulse-rate is not complicated by the action of other factors.

2. The addition of various extracts of the anterior pituitary gland to the medium perfusing type *E* hearts led to a disproportionate increase in pulse rate above 10° C., so that the observed temperature coefficient was increased and curves of type *B* or *D* were produced. The variation in the response obtained by various pituitary extracts has been attributed to quantitative differences.

3. The typical action of anterior pituitary extract was only obtained when the heart was perfused with Ringer solution containing adrenaline. This phenomenon has been explained by assuming that there is a synergistic action between adrenaline and an anterior pituitary hormone which is inhibited at temperatures below about 10° C.

4. Thyroxine was found to have no action on the type *E* heart perfused with Ringer containing adrenaline, but if an extract of anterior pituitary were also present, then the type *E* curve was changed into type *C*. This is in agreement with the work of Carter (1933). Experiments were also made which showed that previous injection of thyroxine into frogs kept in the cold changed type *A* (winter) into type *C* (summer) curves.

5. It was found that adrenaline could either increase, decrease, or have no effect on, the temperature coefficient of the isolated heart. A decrease in the temperature coefficient was only observed in cases where the hearts had been isolated from frogs with active thyroids and has been attributed to the experimental technique employed. The fact that an increase in temperature coefficient may or may not be caused by adrenaline, as well as the fact that type *A* or *C* curves have been obtained when hearts were perfused with adrenaline-free Ringer has been attributed to the persistence, in varying degree, of an active sympathetico-mimetic substance in the heart for at least several hours after isolation.

6. It has been shown that the type *C* temperature-pulse rate curve is the only one which can definitely be attributed to the presence of the thyroid hormone. The seasonal occurrence of this type of curve closely parallels the cycle of thyroid activity described by Sklower (1925) and Meisenheimer (1936).

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STUDIES IN DIURNAL RHYTHMS

I. RHYTHMIC BEHAVIOUR IN MILLIPEDES

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(*Received 7 September* 1950)

(With Six Text-figures)

INTRODUCTION

In recent years the phenomenon of 24 hr. periodicity in animals has attracted considerable attention. Diurnal rhythms have been reviewed by Welsh (1938), Park (1940) and Calhoun (1944), and present many interesting physiological and ecological problems. It is evident that they are common and manifest in diverse ways.

Park, Lockett & Myers (1931), in a short study of nocturnal ecology among forest animals, including the millipede *Polydesmus serratus*, reached some important conclusions: the nocturnal species have a definite rhythm of activity and are inactive by day. The maximum activity is usually in the middle of the night, and tends to increase with increasing relative humidity and decreasing air temperature and rate of evaporation. Conversely, activity decreases with decreasing relative humidity, increasing temperature and evaporation rate. Nocturnal activity was later investigated by means of an aktograph with the large tropical *Spirobolus marginatus* (Park, 1935), and it was found that closely marked cycles of activity persisted during starvation for periods up to 18 days, showing that the activity was not associated with feeding periods or the regular occurrence of hunger rhythms. Although the millipedes were kept in darkness at constant temperature and humidity, the general character of the rhythm did not change, the animals being preponderantly active at night. Therefore Park concluded that *Spirobolus* has an 'inherent nocturnal rhythm'. In his 1940 paper the term 'endogenous activity' was used, and such rhythms were divided into 'inherent' (i.e. genetical) and 'habitual' ones. Calhoun (1944) considered all endogenous rhythms to be of the latter category because the genetical determination of 24 hr. rhythm had in no case been demonstrated.

MATERIAL

Diurnal rhythms have been investigated here in four species of millipedes: *Paradesmus gracilis* (C. L. Koch), a tropical species originating from the East Indies but now widely distributed in temperate regions of Europe and America where it is confined to greenhouses; *Blaniulus guttulatus* (Bosc) the 'Spotted snake-millipede',

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a well-known pest of sugar-beet, potatoes, strawberries, and other agricultural crops (Cloudsley-Thompson, 1950); and two large West African species kindly given to me by Dr Edward Hindle, F.R.S. My thanks are due to Dr F. A. Turk for identifying them as a species of *Ophistreptus* (three specimens), and a new subspecies of *Oxydesmus platycercus* Attems (two specimens).

VISUAL EXPERIMENTS ON BRITISH SPECIES

On account of their small size and light weight, *Paradesmus* and *Blaniulus* are unsuitable animals to use in aktographs. A visual method of estimating activity was therefore used instead. A number of millipedes (ten *Paradesmus* or twenty *Blaniulus*) were placed in a crystallizing dish containing humus, and the number of animals clearly visible on the surface was counted at regular intervals. In this way a measure of their nocturnal activity was obtained. Two identical dishes were employed so that one could be kept in darkness while the other was exposed to light as a control. The results of a number of such experiments are summarized below (Table 1).

Table 1. *Diurnal rhythm of* Paradesmus gracilis

	Dish no. 1				Dish no. 2			
Time ...	05.00 Dark	11.00 Light	17.00 Dusk	23.00 Dark	05.00 Dark	11.00 Light	17.00 Dusk	23.00 Dark
	Control, exposed to daylight				Kept in darkness			
9 days: Total	53	7	37	44	56	28	37	22
Mean	6	1	4	5	6	3	4	2½
	In dark, rhythm reversed				Kept in darkness			
7 days: Total	29	25	23	24	46	33	40	33
Mean	4	3½	3	3½	6½	5	6	5
	In daylight, antennae removed				Kept in darkness			
10 days: Total	66	50	59	73	76	66	65	82
Mean	6½	5	6	7½	7½	6½	6½	8
	Control, exposed to daylight				In daylight, last segment of antenna removed			
10 days: Total	69	42	54	65	99	100	97	96
Mean	7	4	5½	6½	10	10	9½	9½
	In dark, captured at night				Kept in darkness			
20 days: Total	154	130	124	123	81	43	71	61
Mean	7½	6	6	6	4	2	3½	3
	Number feeding on banana				Number feeding on banana			
10 days: Total	25	9	12	19	11	9	15	9
Mean	2½	1	1	2	1	1	1½	1
	In daylight, on asbestos wool				In dark, on asbestos wool			
10 days: Total	31	17	43	30	26	11	14	9
Mean	3	1½	4	3	2½	1	1½	1

Unless otherwise described, the millipedes were freshly captured during the day before each experiment. In one case an attempt was made to reverse the diurnal rhythm by exposing the animals to electric light for 5 nights before the experiment, and keeping them in darkness during the daytime. When antennae or segments of

antennae were removed, 10 days were allowed to elapse before the animals were used in experiments.

From the figures obtained, it is evident that the diurnal rhythm in *Paradesmus* is dependent upon light, being well marked when exposed to daylight, and not apparent in continual darkness. Within the limits of the experiment, however, there was no evidence of persistence of the rhythm for 2 or 3 days in continued light or darkness, as obtains with *Ptinus tectus* (Bentley, Gunn & Ewer, 1941). The importance of light is also seen from the fact that the rhythm was immediately reassumed on exposure to daylight and darkness (Table 2).

Table 2. *Recovery of diurnal rhythm in* Paradesmus gracilis *on exposure to daylight*

	Dish no. 1				Dish no. 2			
Time ...	06.00 Dawn	12.00 Light	18.00 Dusk	24.00 Dark	06.00 Dawn	12.00 Light	18.00 Dusk	24.00 Dark
	Exposed to daylight							
2 days: Mean	8	2½	8	7½	6½	2	6½	8
	Kept in darkness							
10 days: Total	67	52	63	55	49	51	54	54
Mean	6½	5	6½	5½	5	5	5½	5½
	Exposed to daylight							
1 day: Total	6	2	4	7	6	3	7	6

The figures also suggest (Table 1) that removal of the antennary cones only has a more depressing effect than removal of the entire antennae (cf. Cloudsley-Thompson, 1951). The rhythm continued in the absence of food.

Blaniulus guttulatus also exhibited a diurnal rhythm largely dependent upon light (Table 3).

Table 3. *Diurnal rhythm in* Blaniulus guttulatus

	Dish no. 1				Dish no. 2			
Time ...	08.00 Light	12.00 Light	18.00 Dusk	24.00 Dark	08.00 Light	12.00 Light	18.00 Dusk	24.00 Dark
	Control, exposed to daylight				In daylight			
5 days: Total	26	20	35	44	17	15	27	32
Mean	5	4	7	9	3½	3	5½	6½
					In darkness			
10 days: Total	49	39	55	72	86	111	101	89
Mean	5	4	5½	7	8½	11	10	9
					In daylight			
5 days: Total	18	13	31	39	21	25	55	50
Mean	3½	2½	6	8	4	5	11	10

In each crystallizing dish were placed twenty *Blaniulus* where only ten *Paradesmus* had previously been used. Yet the figures for *Blaniulus* are not nearly twice as great

as those for *Paradesmus*. This confirms the fact, readily noticeable in the field, that there is a much greater tendency for *Paradesmus* to come to the surface, than for *Blaniulus* to do so.

The results furnish an interesting confirmation of the parallel evolution which has taken place in the Diplopoda and Chilopoda. Salt, Hollick, Raw & Brian (1948) have shown that the attenuated Geophilidae are found at deeper levels in the soil than Lithobiidae which bear a superficial resemblance to *Paradesmus*. It is evident that the elongated shape of Blaniulidae and Geophilidae is correlated with a more subterranean habitat.

It has already been shown that sudden drops in temperature engender an outburst of activity in millipedes (Cloudsley-Thompson, 1949, 1951). It seemed probable, therefore, that the drop in temperature which occurs at sunset, might play a part in diurnal rhythms.

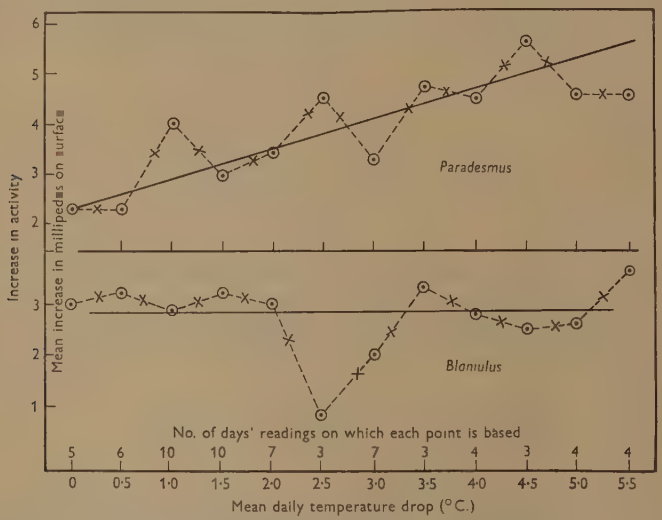

Fig. 1. Relationship between activity and falling temperature in *Paradesmus* and *Blaniulus*. ⊙, the mean result; ×, first approximation.

This was investigated by counting the number of *Paradesmus* and *Blaniulus* visible on the surface at 08.00, 14.00, 20.00 and 24.00 hr., and noting the temperature at the same time. The mean of the first two sets of readings was then subtracted from that of the second two: the difference in the number of millipedes on any particular day (i.e. the increase in activity in the evening) could then be related to the fall in temperature on that day. The result of a number of these readings of ten *Paradesmus* and twenty *Blaniulus* over a period of ten weeks is shown in Fig. 1. In the case of *Paradesmus* there is a definite correlation between the amount of activity and the drop in temperature. It is probable that in *Blaniulus* the number of animals on the

surface is less representative of their state of activity on account of their subterranean habit.

AKTOGRAPH EXPERIMENTS ON TROPICAL SPECIES

Both *Ophistreptus* and *Oxydesmus* are heavy enough to operate a large aktograph, and it was possible to make experiments extending over periods of several days. The arena of the aktograph was oval in shape and measured 12 × 6 in. It was lined with damp humus so that the animal was not affected by starvation and other discomforts as in the experiments of Park (1935). The humidity must have approached 100 %. A fin at the end of a rod from the bottom of the arena reaching into an oil-bath acted as a damper, while the position of weights on the rod could be adjusted to give maximum sensitivity. The balance could be adjusted by moving a small weight on the rod counterbalancing the writing needle, and the arena was pivoted on two steel bolts ground to a fine point.

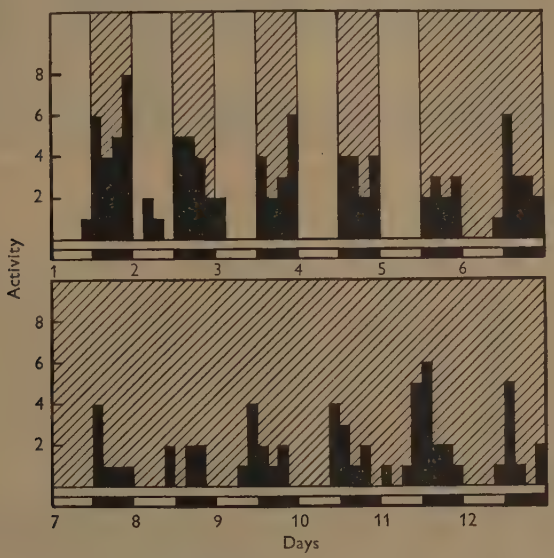

Fig. 2. Activity of *Ophistreptus* at room temperature, at first under natural lighting, and later in darkness.

The smoke drum records were analysed graphically by means of block histograms, the activity during each 3 hr. period being expressed by the number of times that the animal crossed the axis, and rocked the arena of the aktograph. Fig. 2 shows that the rhythmic activity of *Ophistreptus* at room temperature became less marked when natural lighting was replaced by continuous darkness. A 24 hr. rhythmic cycle could be discerned, however, up to 19 days under constant conditions of temperature (21·5° C.) and lighting (60 W. at 2 ft.) (Fig. 3). This compares with periods up to 18 days during which similar cycles of activity under constant

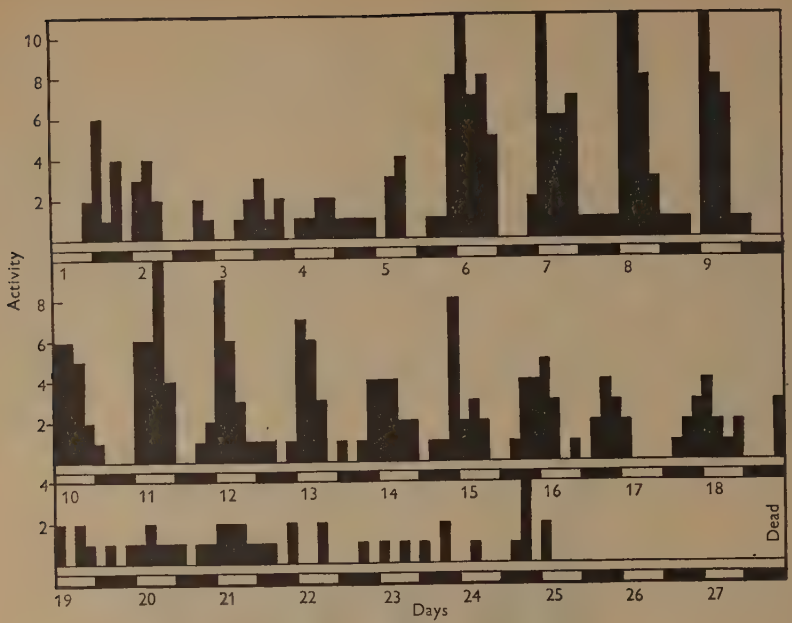

Fig. 3. Persistence of rhythmic activity in *Ophistreptus* in constant temperature (21·5° C.), and lighting (60 W. at 2 ft.).

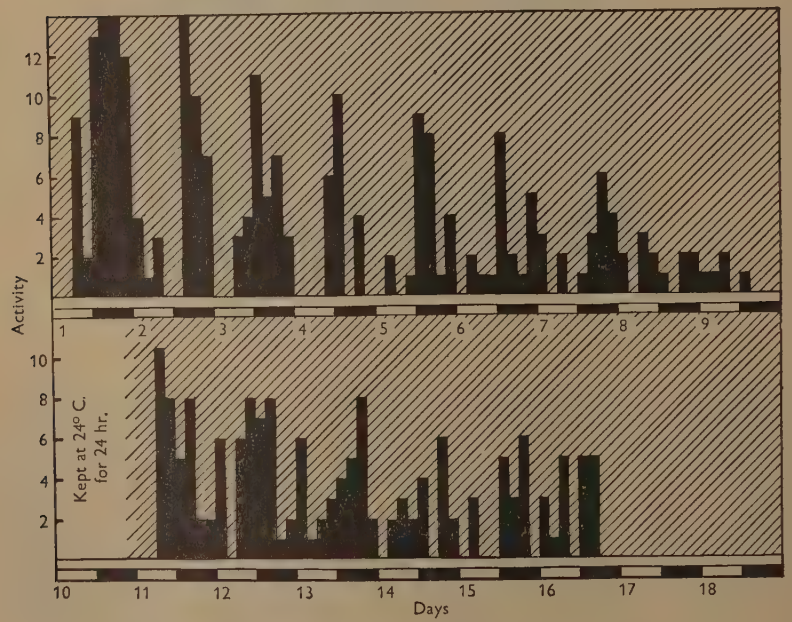

Fig. 4. Persistence of rhythmic activity in *Oxydesmus* in constant temperature (21·5° C.) and darkness, stimulated by the drop in temperature following 24 hr. at 24° C.

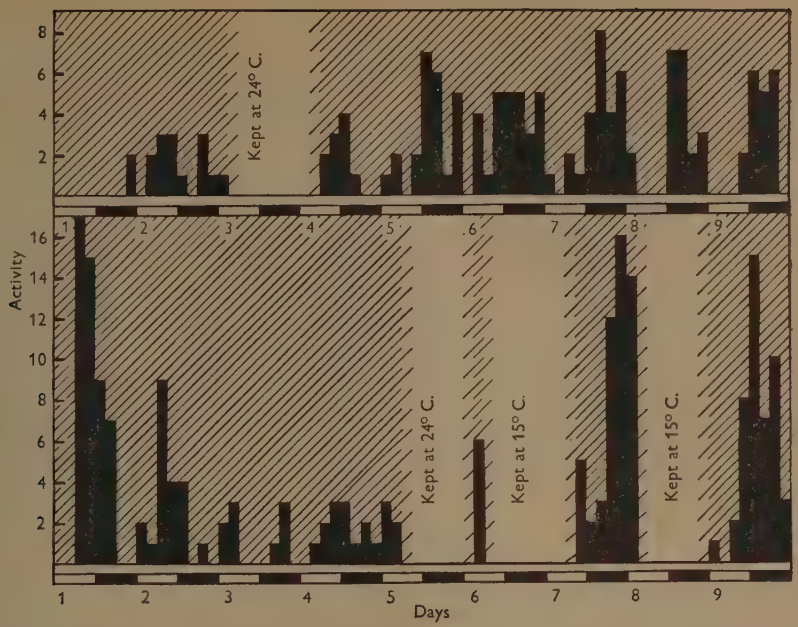

Fig. 5. Effects of periods of increased and decreased temperature on the activity of (*a*) *Ophistreptus*, (*b*) *Oxydesmus*, in constant temperature (21·5° C.) and darkness.

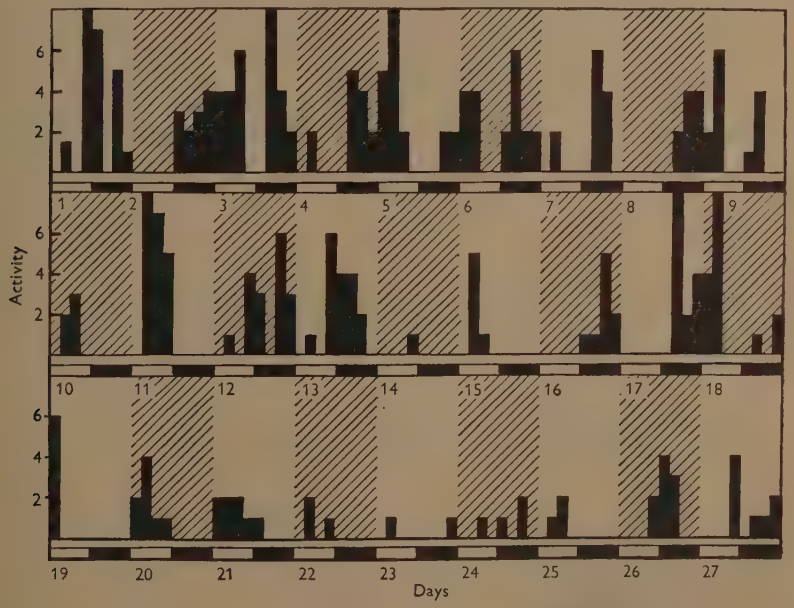

Fig. 6. Effect of alternating 24 hr. periods of light (60 W. at 2 ft.) and darkness, on the activity of *Ophistreptus* at 21·5° C. The animal had previously been kept in constant temperature and darkness for 14 days.

conditions have been recorded in *Spirobolus* (Park, 1935). A persistent rhythmic activity was also demonstrated in *Oxydesmus* (Fig. 4). In addition, this experiment illustrated the effect of falling temperature in stimulating the rhythm.

The fact that either a drop, or an increase, in temperature engendered an outburst in activity in *Ophistreptus* is shown by Fig. 5. The arena of the aktograph was placed in constant temperature rooms at 24° or 15° C. for approximately 24 hr. before being returned to 21·5° C. In each case the change in temperature was followed by an outburst in activity which was however more marked when the temperature was increased, perhaps because at 15° C. the animals were quite immobilized by the cold, and in any case the temperature change was greater. Constant temperatures over a long period appeared to have a depressing effect on the animals.

The diurnal cycle of quiescence and activity was not affected by alternating 24 hr. of darkness with 24 hr. of light (60 W. at 2 ft.) (Fig. 6). Perhaps in their natural gloomy habitat in tropical forest, light is an insignificant environmental factor. Hence it appears probable that temperature fluctuations are of primary importance in the initiation and maintenance of diurnal rhythms in these species.

SUMMARY

Visual experiments on two small British species of millipedes have demonstrated that the diurnal cycle of rhythmic activity as represented by the number of animals on the surface is primarily a response to light and darkness, but is also correlated with the stimulus of falling temperature in the evening.

Aktograph experiments on two large West African species of millipedes have demonstrated an endogenous diurnal rhythm independent of fluctuating light and temperature, and persisting (in *Ophistreptus*) up to 19 days. Locomotory activity is stimulated both by increases and decreases of temperature; and it is probable that temperature fluctuations are of primary importance in the initiation of diurnal rhythms. The effect of light on activity is slight, but constant temperatures over long periods have a depressing effect.

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THE VARIATION IN FAT AND GLYCOGEN CONTENT OF THE BOT FLY (*GASTROPHILUS INTESTINALIS*) LARVA TRACHEAL ORGAN DURING DEVELOPMENT

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(*Received* 8 *September* 1950)

(With Two Text-figures)

Numerous analyses on the chemical composition of various insects have shown that, as in mammals, fat and glycogen constitute the principal food reserves. Among holometabolic insects the accumulation of these two substances during the larval instars is, to a varying extent, utilized during the process of pupation. Most of the data on the chemical changes during metamorphosis are summarized by Needham (1942). However, as pointed out by Wigglesworth (1939), analyses of the body as a whole show only the gross alterations, and almost nothing is known concerning the composition of the separate organs. The only information available concerns the glycogen content of the isolated insect fat body; thus, Babers (1941) found that in the mature larva of *Prodenia eridania* (Lep.) the glycogen formed 23·3% of the fat body dry weight, while Yokoyama (1934) estimated the glycogen content of the silkworm fat body to vary from 2 to 17% of the dry weight according to age. No figures have been published for the fat content of the fat body, but histological evidence (Pardi, 1939; Wigglesworth, 1942) clearly demonstrates that fat is also present in high concentration.

In the present work the changes in fat and glycogen occurring in the tracheal organ cells of *Gastrophilus intestinalis* de Geer during certain stages of development have been followed quantitatively. A description of the life cycle of *Gastrophilus* is given in earlier papers (Dinulescu, 1932; Keilin, 1944; Levenbook, 1950*a*), but it may briefly be mentioned that late 2nd instar and 3rd instar larvae up to the time of pupation live attached to the gastric mucosa of the horse's stomach. The duration of the 3rd instar lasts some 7–8 months, from November to June, and the greater part of this period is spent in a diapausing condition. From July onwards the larvae leave the alimentary tract and pupate in the soil.

The tracheal organ consists of a bunch of very large cells occupying the posterior portion of the body. The structure of these cells, and their relation to the respiratory system, may be found in the works of Enderlein (1899), Prenant (1900), Portier (1911), Radu (1932) and Keilin (1944). As first shown by Dinulescu (1932) and confirmed by the present author, the cells are developed from undifferentiated fat body cells during the first larval instar. Apart from the possession of haemoglobin (Keilin & Wang, 1946) and intracellular tracheoles, the tracheal cells histologically appear similar to the normal fat body cells present in the more anterior part of the body,

particularly so far as the distribution of fat and glycogen are concerned. Moreover, the one type of tissue merges imperceptibly into the other, and it would appear likely that the storage function of the tracheal and fat body cells are also similar.

MATERIAL AND METHODS

As described in previous papers (Levenbook, 1950*a*, *b*), fresh *Gastrophilus* larvae were obtained from a local knackery and were transported to the laboratory on ice. During June and July the pupae were obtained by placing larvae in a dish containing damp sawdust kept at 24° C.

The washed and dried larvae were bled, cut open longitudinally from the anterior end along the ventral side, and the tracheal organ clearly exposed. This was then cut out, quickly rinsed in ice cold '*Gastrophilus* saline' (Levenbook, 1950*a*) and superficial moisture removed with filter-paper. The tracheal organ thus obtained consisted of the tracheal cells and the chitinous spiracular end-plate, together with the four tracheal trunks which originate from it. A very small portion of the gut and Malpighian tubules was sometimes also included. The tracheal organs from the pupa did not include the spiracular end-plate nor, after the 2nd day of pupation, the intracellular tracheoles, since these had been extruded. However, depending upon the pupal age, a varying number of phagocytes was included with the tracheal cells.

Glycogen was estimated by a method based on that of Good, Kramer & Somogyi (1933). The cold, dried, tracheal organs were dropped into tared, stoppered tubes containing 20% (w/v) KOH at 100° C. After a few minutes the tubes were cooled and reweighed, and hence the weight of tissue calculated. The tubes were then replaced in the boiling water-bath for a further 30 min. or longer. The chitinous material present was not dissolved by this treatment, and formed a gelatinous layer on top of the alkali. The solution was cooled, quantitatively filtered through washed glass-wool, and the glycogen in the filtrate determined. Reducing value was measured by the method of Miller & Van Slyke (1936).

For estimation of total fatty acids + sterols, hereafter referred to as total fat, 1–1·5 g. of tracheal organs were ground to a smooth paste with washed sand and a few drops of water, and this paste extracted for 24 hr. at room temperature with 50 ml. 3:1 (v/v) ethanol-ether (Boyd, 1936). Both solvents were redistilled, the ether being treated just before use so as to destroy peroxides. The extract was filtered through fat-free filter-paper, the filtrate again made up to 50 ml., and 5–10 ml. aliquots taken for analysis of total fat (Bloor, 1928) or phospholipid (Bloor, 1937). To obtain satisfactory blank values it was necessary to purify the Analar petroleum ether as described by Bloor (1928), and to ensure scrupulously clean glassware.

The dry weight of the tissue was estimated after drying in an oven at 105° C. to constant weight. The chitinous material present in the larval tracheal organ accounted for 2–3% of the total wet weight which, for mature 3rd instar larvae, was about 50 mg.

RESULTS

Glycogen. Kemnitz (1916) found that the material he assumed to be *Gastrophilus* glycogen as judged by stability to alkali, precipitation by ethanol, and reducing properties following acid hydrolysis, had an optical rotation $[\alpha]_D^{20°} = +192{\cdot}6°$ C., which is within the range of 190–200° C. given by Bell (1948) for glycogen. Conclusive additional evidence for the presence of glycogen in the tracheal cells was obtained as follows.

One gram of tracheal organs was dissolved in hot KOH and the precipitate obtained by the addition of 1·5 vol. ethanol was dissolved in warm water and reprecipitated twice more with ethanol. After drying *in vacuo* it had the following properties. It was readily soluble in warm water, and this solution gave a red-brown colour with iodine; no such colour was obtained after an aliquot had been incubated with salivary amylase. The solution had only very weak reducing properties before acid hydrolysis, whilst after hydrolysis there was a marked increase in reducing value (expressed as glucose) when measured by the reduction of alkaline ferricyanide, and the value so obtained was the same as when the actual glucose content of the hydrolysate was measured with glucose oxidase (Keilin & Hartree, 1948). The Seliwanoff reaction for fructose was negative (cf. Levenbook, 1950*a*), and hence the tracheal organ glycogen is composed of the usual D-glucose units.

It is relevant in this connexion that substances are present in *Gastrophilus* larva blood (Levenbook, 1950*a*) and in *Lucilia sericata* pupae (Evans, 1932), which are also alkali stable, ethanol precipitable and have reducing properties following acid hydrolysis, but which, judged by the additional criteria presented above, are not true glycogen.

The glycogen content of the tracheal cells from larvae of different ages was determined at fortnightly intervals during two generations. Two to three samples of about five tracheal organs were used to obtain a single value, and the combined results are shown in Fig. 1.

The glycogen content was lowest in the tracheal organ from fully grown 2nd instar larvae—the earliest stage examined, and there was a considerable increase in 3rd instar larvae immediately after the 2nd moult. This would indicate that little if any glycogen had been utilized in the formation of new chitin. The glycogen content during the 3rd instar did not change greatly from October to the beginning of December, but thereafter a marked, although temporary, increase in glycogen occurred. From the middle of December the glycogen content rose continuously to reach a maximum value of just over 40% of the total dry weight in March, after which there was a gradual decrease up to the time of pupation. During the initial stages of pupation glycogen was rapidly utilized, but as metamorphosis continued the rate of glycogen consumption gradually declined.

It should be noted that the absolute values for both glycogen and fat after the 4th day of pupation are probably not as accurate as those for the earlier stages; by about the 4th day phagocytosis of the tracheal organ has proceeded to the extent that it becomes difficult to separate the tracheal cells from the adjoining tissues,

while after the 10th day the inside of the pupa becomes thick and creamy and, although numbers of separate tracheal cells still remain, the tracheal organ as such no longer exists.

Fat. The fat content of the tracheal cells was also measured at fortnightly intervals during two generations, replicate determinations on twenty to thirty pooled tracheal organs serving to yield a single value. The results are shown in Fig. 2.

No difference was found between the fat content of the tracheal organ from late 2nd and earliest 3rd instar larvae, and there was a progressive accumulation of fat from the earliest stage to the 10th day of pupation. The amount of fat increased most rapidly during the first 7 days of pupation, while during early December, and to a lesser extent again in February, there appeared to be an abrupt fall and rise.

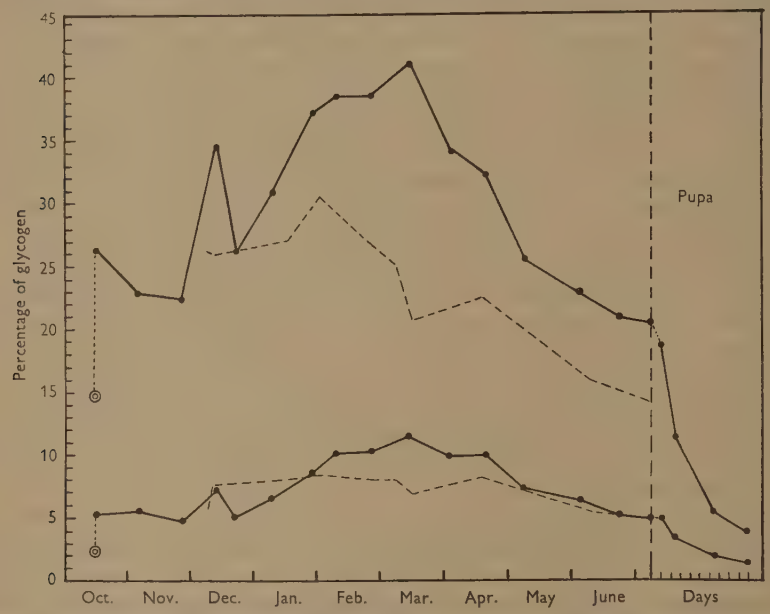

Fig. 1. The variation in the glycogen content of the *Gastrophilus* tracheal organ. Circles = 2nd instar, dots = 3rd instar larvae. Upper and lower curves are based on dry weight and wet weight respectively. Dotted curves are for the glycogen content of the whole larva calculated from the data of Kemnitz (1916).

Phospholipid. The phospholipid content of the tracheal cells was measured during the 3rd instar at intervals of 3–4 weeks. As noted by other workers (e.g. Artom, 1932), the phospholipid content, as measured by the phosphorus content of the ethanol-ether extract employing a multiplication factor of 25, was considerably higher than that fraction of the total lipid which was relatively insoluble in acetone and precipitable with $MgCl_2$, i.e. by the Bloor (1937) procedure. This discrepancy may mean that lecithin is not a major component, but nothing appears to be known

about the constitution of insect phospholipids. The data obtained by the Bloor method are shown in Table 1.

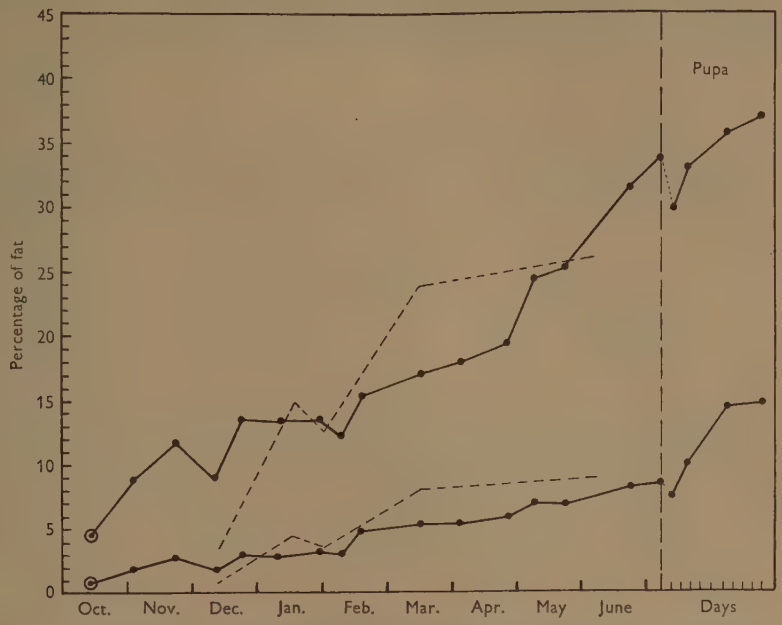

Fig. 2. The variation in the fat content of the *Gastrophilus* tracheal organ. Circles = 2nd instar, dots = 3rd instar larvae. Upper and lower curves are based on dry weight and wet weight respectively. Dotted curves are for the glycogen content of the whole larva calculated from the data of Kemnitz (1916).

Table 1. *Phospholipid content of the 3rd instar* Gastrophilus *tracheal organ*

Date	Phospholipid (% of wet wt.)	Date	Phospholipid (% of wet wt.)
10.i.47	2·4	7.v.48	1·7
17.ii.47	2·1	25.v.47	1·3
19.ii.48	2·1	24.vi.47	0·99
14.iii.47	2·0	8.vii.48	0·87
20.iv.47	2·2	20.vii.47	0·78
2.v.47	1·9		

On a wet-weight basis the tracheal organ phospholipid was about three times greater at the beginning of the 3rd instar than just before pupation. However, during the same period the tissue dry weight increased by about one-third of its original value, so that expressed in absolute figures the phospholipid decreased from January to July by about 50%.

Tracheal organ fat and glycogen during starvation. Kemnitz (1916) investigated the changes in fat and glycogen of fasting *Gastrophilus* larvae both in the presence

and absence of oxygen. He showed that both aerobically and anaerobically there was a decrease in glycogen and an increase in fat but, surprisingly, more glycogen was consumed in the presence of O_2 than in its absence.

An examination of the tracheal organ of fasting *Gastrophilus* larvae has shown that the changes referred to above for the whole larva are here seen to occur to an exaggerated degree (Table 2).

Table 2. *Changes in tracheal organ fat and glycogen in fasting* Gastrophilus *larvae shortly before pupation. The larvae were kept in a dish containing slightly acidified tap water.*

	Glycogen (mg./g. wet wt.)	Fat (mg./g. wet wt.)
Fresh larvae	53·2	87·9
Larvae 5 days old	36·9	123·2
Net change	−31%	+40%

The O_2 uptake of larvae immersed in a shallow layer of water is a little less than in air (Levenbook, unpublished), but is presumably at least as high as in their normal environment, especially since the O_2 tension in the horse's stomach is said to approach zero (Tappeiner, 1883). The changes in Table 2 therefore indicate that, due to incomplete aerobic oxidation, only a part of the available energy stored as carbohydrate is utilized—a type of reaction discussed in some detail for invertebrates by Brand (1946).

DISCUSSION

Since the tracheal organ contributes a major portion of the weight of the organized tissue of the *Gastrophilus* larva, it might be expected that the changes which it shows in fat and glycogen would be also manifested in the variation of these substances for the whole larva.

Considering first the glycogen, Dinulescu (1932) analysed 2nd and 3rd instar *Gastrophilus* larvae of mixed species, and found that the lowest values (3% of the larval wet weight) occurring in the 2nd instar were followed by a continuous increase in glycogen during the 3rd instar, with a maximum of 9–10% just before pupation. Kemnitz (1916), on the other hand, found that maximum storage of glycogen took place at the end of January, and that thereafter glycogen was progressively consumed. Fig. 1 shows that the general trend observed by Kemnitz (1916) is in agreement with the present data for the glycogen content of the tracheal organ. Furthermore, the rest of the larval tissues other than the tracheal organ must also be rich in glycogen, since the values on a wet-weight basis for the whole larva and the isolated tracheal cells are in fairly good agreement. Expressed on a dry-weight basis, the tracheal organ values are considerably higher, no doubt because they do not include the considerable amount of water in the larval haemolymph.

The sudden increase in tracheal organ glycogen and the simultaneous decrease in fat that apparently occur during December, is in each case due to a single value (see

Figs. 1 and 2) which, although the mean of a number of samples from two generations, does not exclude the possibility of a sampling error. However, the fact that the changes for both substances occur at the same time, and that Kemnitz (1916) found a similar sudden decrease in fat for the whole larva, suggests that these fluctuations are genuine. But at present no explanation can be offered as to their significance.

According to Dinulescu (1932) *Gastrophilus* larvae during the greater part of the third instar take very little, if any, nourishment; the progressive decrease in glycogen and the concomitant increase in fat which occurs from the end of February onwards, the inverse fluctuations in these substances discussed above, and the decrease in glycogen and increased fat in the tracheal organ of larvae fasting *in vitro*, all suggest that glycogen can be converted into fat in the tracheal cells. Although experimental proof for the occurrence of this reaction in insects' tissues is at present lacking, the tracheal organ would appear particularly suitable for testing this hypothesis *in vitro*.

Gastrophilus fat is a yellowish oil, containing a considerably higher proportion of saturated fatty acids (iodine number = 70·2, saponification value = 200·5) than is found in the majority of insects (Timon-David, 1930), but similar to that found by Rainey (1938) for *Lucilia sericata*. A high percentage of unsaturated fatty acids is characteristic of insect fats, and the low iodine number of *Gastrophilus* fat might be due to either a low iodine number of the food fats ingested (Yuill & Craig, 1937), or to the relatively high temperature (37–38° C.) of the larval environment, since Fraenkel & Hopf (1940) found the phosphatide fatty acids of certain Dipterous larvae became increasingly saturated as the temperature was raised to 35°.

Although fat as measured in the present work includes only total fatty acids plus sterols, there is good reason to suppose that this fraction accounts for the major bulk of the total fat (cf. Wigglesworth, 1939; Rainey, 1938; Finkel, 1948). As in many other insects, the amount of fat accumulated by *Gastrophilus* increases throughout larval life, and this is reflected in the fat content of the tracheal organ; by the 10th day of pupation fat accounts for some 36% of the dry weight of the tissue. By analogy with all other insect pupae, this large fat reserve is subsequently utilized during development of the imago. The time at which fat is consumed by Diptera during metamorphosis appears to vary with different genera; in *Lucilia* fat already decreases in the late 3rd instar larva (Evans, 1932), whereas in *Calliphora* there is no such decrease until the second half of the pupal period (Frew, 1929).

The small amount of fat in the tracheal cells of early 3rd instar larvae is almost entirely accounted for by phospholipid; since the amount of the latter gradually decreases while the total fat increases, towards the end of the instar phospholipid finally constitutes $< 10\%$ of the total fat. In the mealworm also, as fat accumulates, so the percentage of phospholipid declines (Teissier, 1931; Finkel, 1948). The reason for this inverse relationship is obscure, but it may be that energy is better stored in the fatty acids of the relatively stable neutral triglyceride than of the more soluble and reactive phospholipid. It remains to be seen whether phospholipid is reformed during the later stages of pupation when the neutral fat is mobilized.

SUMMARY

1. In the tracheal organ cells of *Gastrophilus* fat and a material shown to be glycogen are accumulated as food reserves; the amounts of these substances in the tracheal organ have been followed quantitatively during development.

2. The glycogen content of the tracheal cells is low during the 2nd and early 3rd instars, gradually increases to a maximum of over 40% of the tissue dry weight during the middle of the 3rd instar, and then progressively declines.

3. Fat is accumulated in the tracheal cells throughout development, and attains a maximum of over 35% of the tissue dry weight at about the 7th–10th day of pupation.

4. At the beginning of the 3rd instar phospholipid accounts for almost the whole of the total fat; at the end of the instar it forms $<10\%$ of the total fat.

5. In the tracheal organ of fasting *Gastrophilus* larvae, glycogen is probably converted into fat.

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THE EFFECT OF CARBON DIOXIDE AND CERTAIN RESPIRATORY INHIBITORS ON THE RESPIRATION OF LARVAE OF THE HORSE BOT FLY (*GASTROPHILUS INTESTINALIS* DE GEER)

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(*Received* 8 *September* 1950)

(With Three Text-figures)

In a previous paper (Levenbook, 1950*a*) it has been demonstrated that the larvae of the fly *Gastrophilus intestinalis* de Geer, parasitic in the stomach of the horse, contain far more carbon dioxide in their haemolymph and tissues than is normally found in insects, and that this is a consequence of the extremely high CO_2 tension prevailing in their normal habitat. In the present study it will be shown that these larvae are so adapted to CO_2 in their environment that its removal leads to a profound depression of their respiration and to eventual death.

The respiration of *Gastrophilus* larvae has been investigated by Kemnitz (1916), Dinulescu (1932) and, in unpublished work to be described below, by Keilin. Kemnitz was principally interested in overall metabolic changes, and his data are difficult to interpret in terms of oxygen uptake per unit weight of larva. In addition, since his experiments were continued over a number of days, it is almost certain that part of the gas changes which he measured was due to bacterial contamination. Dinulescu's data, on the other hand, are subject to a number of technical criticisms; these include insufficient attention to adequate temperature control, experiments of only short duration and, most important of all, no measurements in the presence of CO_2. Within the limits of these qualifications, however, he found that the respiratory rate was highest in 2nd instar larvae and lowest during the long quiescent period (diapause) which occurs during the 3rd instar, while keeping the larvae for any length of time '*in vitro*' produced a progressive decrease in the rate of O_2 uptake.

References to the life cycle, morphology and bionomics of the *Gastrophilus* larva may be found in the works of Dinulescu (1932), Keilin (1944) and Keilin & Wang (1946), and hence attention need only very briefly be drawn to two peculiar respiratory adaptations in the larva of importance for the present work.

The first of these concerns the mass of giant tracheal cells at the posterior end of the body forming the so-called tracheal organ. These cells are rich in haemoglobin, the properties of which were studied by Keilin & Wang (1946), and are abundantly supplied with intracellular tracheoles. The actual importance of the tracheal organ in respiration is yet to be investigated, but from the available evidence it would appear likely that it functions as a primitive 'lung', especially at low oxygen tensions. Secondly, in the 2nd and 3rd instars, the post-abdominal spiracles lie in a depression or pouch formed by transverse dorsal and ventral folds or lips of the larval cuticle.

These lips exert a regulatory function in respiration, since they can be expanded as a result of muscular pressure transmitted through the larval haemolymph and, in thus completely sealing off the spiracle, they effectively prevent any spiracular gas exchange. It may be noted that a spiracular closing mechanism is relatively rare among Dipterous larvae, and when present is usually indicative of an aquatic or semi-aquatic habitat, e.g. larvae of Culicidae, Bibionidae, Psychodidae, etc.

MATERIAL

Mature, 3rd instar *G. intestinalis* larvae were obtained from a local knackery. As soon as possible after the killing of an infected host, and still attached to the stomach, they were transported to the laboratory in a thermos flask containing water at 38° C. After washing the stomach with warm water, the larvae were carefully picked off the mucosa with forceps, rinsed in warm water, and either used immediately, or kept in a large dish containing slightly acidified tap water at room temperature. Larvae which had been kept warm continuously and were employed for an experiment within an hour after their removal from the horse will be referred to as fresh larvae, while those kept for 1 or 2 days as 24 or 48 hr. larvae respectively.

Except where stated otherwise, all experiments were carried out on diapausing larvae obtained between the beginning of November and the end of May.

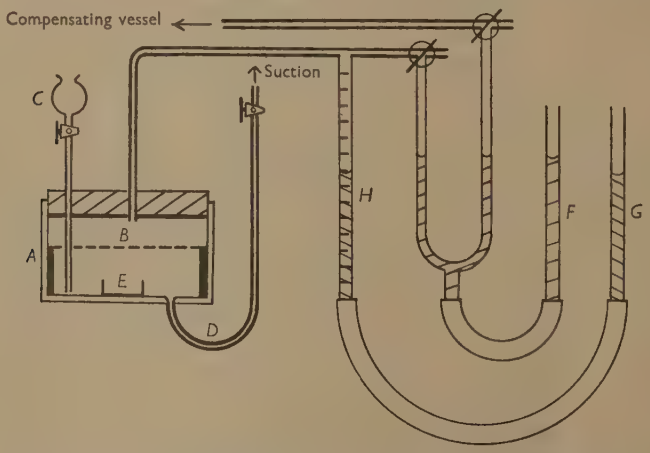

Fig. 1. Diagram of Haldane manometric apparatus for measurement of insect respiration in presence of CO_2.

METHODS

The majority of the present experiments were carried out with a modified Haldane manometric apparatus which is shown diagrammatically in Fig. 1.

A circular flat-bottomed glass vessel *A*, approximately 6 cm. in diameter and 3 cm. in depth, was made with side indentations on which rested a stainless steel gauze *B*. A small funnel *D*, blown into one side of the base, was connected to a water suction

pump through a piece of wide bore capillary tubing and a glass tap. A small centre well *E* to contain water was sealed on to the bottom of the vessel. This vessel was closed with a well-fitting paraffin-waxed rubber bung, on to the lower surface of which was cemented a thin Perspex disk to prevent the larvae from attaching themselves to the rubber.

A small thistle funnel *C*, made of wide bore capillary tubing and fitted with a glass tap, passed through the rubber bung and Perspex disk to within 1–2 mm. of the bottom of the vessel, while a narrow bore capillary tube inserted into the bung connected to a Haldane (1920) constant pressure manometer. The latter was slightly modified in that the normal 1 ml. graduated pipette was replaced by a 5 ml. pipette, *H*. The other limb of the manometer led to a compensating flask of the same size as the experimental vessel, and contained approximately 9 ml. water. Both vessels were immersed in a thermostatically controlled constant temperature water-bath.

The procedure for carrying out a measurement was as follows. 0·5 ml. water was put into the small centre well, and twenty weighed *Gastrophilus* larvae were placed on the steel gauze disk. The apparatus having been assembled, both vessels were clamped in position in the water-bath with all taps open. By means of gentle suction at *D*, a slow stream of air was passed through the experimental flask for 90 min. to allow of temperature equilibration and settling down of the larvae. (This time was empirically determined as being the minimum required in order subsequently to obtain fairly constant readings. The liquid level in the graduated pipette was set at a convenient height by raising or lowering *G*, the water pump turned off, and the manometer taps were turned so as to connect each side with its corresponding vessel. The fluid level in the U-tube having been levelled by means of *F* only, the tap at *D* was closed and a reading taken of the meniscus in the pipette.

The larvae were now allowed to respire for a known period of time, generally 1 hr., after which the level of the liquid in the U-tube was again levelled by means of *F*, and a second reading taken on the pipette. A known volume—3 ml. was a convenient amount—of 2N-KOH previously warmed to 37° C. was then run into the bottom of the vessel through *C*, leaving that part of the funnel between the tap and the end full of alkali so as to prevent the entry of air. The volume of air introduced initially in running in the KOH was negligible. The experimental vessel was then very gently shaken for exactly 5 min., this time having been found sufficient in pilot experiments for the absorption of the respiratory carbon dioxide, and a third reading taken on the pipette.

If the KOH were allowed to remain in the flask, the subsequent rate of O_2 uptake in the absence of CO_2 could now be measured directly. Alternatively, by opening the tap at *C* and applying suction at *D*, the alkali could be removed, any remaining traces being neutralized by running through a little dilute acid from the thistle funnel. The acid was followed by distilled water and a current of air. After a short period of re-equilibration, further measurements could again be made in the presence of CO_2, without the larvae having in any way been disturbed due to handling or shaking throughout the procedure.

In order to calculate the results, it is necessary to take into account the change in

reading on the pipette due to the introduction of a volume of liquid equal to that of the KOH, and also the oxygen utilized during the 5 min. during which the CO_2 was absorbed. The assumption is made that the rate of O_2 uptake during these 5 min. is the same as for the preceding hour. The equations for the conversion of the pipette readings into values for the O_2 uptake and CO_2 production were derived as follows.

Adopting the usual convention that all gas evolved produces a positive, and all gas absorbed a negative reading, then if

x = amount of O_2 absorbed in time t_1,

y = amount of CO_2 evolved in time t_1,

and h_0 = initial pipette reading,

h_1 = pipette reading at time t_1,

h_2 = pipette reading at time t_2 after absorption of CO_2,

Δh = change in pipette reading due to addition of KOH,

$$x+y=h_1-h_0, \tag{1}$$

or
$$x\frac{(t_2-t_1)}{t_1}+y\frac{(t_2-t_1)}{t_1}=\left(\frac{t_2-t_1}{t_1}\right)h_1-h_0, \tag{2}$$

since on the addition of KOH all the CO_2 is absorbed, and thereafter only O_2 uptake is measured,

$$-y+x\frac{(t_2-t_1)}{t_1}=h_2-h_1-\Delta h; \tag{3}$$

subtracting (2) from (3),

$$-y-y\frac{(t_2-t_1)}{t_1}=h_2-h_1-\Delta h-\left(\frac{(t_2-t_1)}{t_1}h_1-h_0\right),$$

or
$$y=\frac{-\left[h_2-h_1-\Delta h-\left(\frac{(t_2-t_1)}{t_1}h_1-h_0\right)\right]}{\left(1+\frac{t_2-t_1}{t_1}\right)},$$

and x (from equation (1)) $=h_1-h_0-y$,

The barometric pressure was noted and these values, corrected to N.T.P. and $t_1=60$ min., were divided by the weight of the larvae to give the required figures for the Q'_{O_2} and Q'_{CO_2}, expressed throughout this paper as μl. of gas per mg. wet weight per hour. This procedure will be referred to below as the Haldane manometric method.

The *protocol* of an actual experiment may be cited as an example.

Wet weight of twenty *Gastrophilus* larvae = 8·597 g.

Barometric height = 765 mm. Hg, temp. = 37° C.

$t_1 = 60$ min.,

$t_2 = 65$ min.,

$h_0 = 2{\cdot}35$ ml.,

$h_1 = 1{\cdot}835$ ml.,

$h_2 = 2{\cdot}97$ ml.,

Δh (for 3 ml. KOH) $= 2{\cdot}75$ ml.,

$$y = \frac{-[2{\cdot}97 - 1{\cdot}835 - 2{\cdot}75 - (\frac{1}{12} \times 1{\cdot}835 - 2{\cdot}35)]}{(1 + \frac{1}{12})}$$

$$= -[1{\cdot}615 - (0{\cdot}043)]\,\tfrac{12}{13}$$

$= 1{\cdot}450$ ml. CO_2 evolved,

$$x = 1{\cdot}835 - 2{\cdot}35 - 1{\cdot}450$$

$= -1{\cdot}965$ ml. O_2 absorbed.

Then $$Q'_{O_2} = \frac{-1{\cdot}965}{8597} \times \frac{765}{760} \times \frac{273}{310} = -0{\cdot}20/\mu\text{l.}/O_2/\text{mg./hr.},$$

$$Q'_{CO_2} = 0{\cdot}15/\mu\text{l.}/CO_2/\text{mg./hr.}, \quad \text{R.Q.} = 0{\cdot}75.$$

The Haldane manometric method was unsuitable for experiments on single larvae, and for this purpose Warburg manometers were used. The technique of Dickens & Šimer (1931) was adapted for use with *Gastrophilus* larvae as follows.

A single weighed larva and 0·05 ml. water were placed in the centre well of a normal type Warburg flask of *c.* 16 ml. capacity. The main compartment contained 2 ml. M/5 potassium permanganate in M/500 H_2SO_4, the side bulb 0·2 ml. of 30% sodium iodide (acidified to N/500 H_2SO_4 just before use). The larva was allowed to respire for a known period of time, after which the two solutions were mixed, resulting in the production of a highly alkaline mixture which on shaking the manometer at 100 oscillations per min. rapidly absorbed the respiratory CO_2 (Krebs, 1930).

In calculating the results for this modified Dickens & Šimer technique a correction had to be applied for the small positive pressure produced in the manometer as a result of mixing the solutions; the value for this was obtained by using a control manometer containing only these two solutions. The assumption is made that the rate of O_2 uptake during the time allowed for absorption of CO_2—generally 5 min.—is the same as for the preceding period. The results are calculated as follows; if

x is the O_2 absorbed in time t_1,

y is the CO_2 evolved in time t_1,

h_0 is the initial manometer reading,

h_1 is the manometer reading at time t_1,

h_2 is the manometer reading after mixing the solutions at time t_2, corrected for the control,

k_{O_2} and k_{CO_2} are the vessel constants for O_2 and CO_2 respectively, then

$$h_1 - h_0 = \frac{y}{k_{CO_2}} + \frac{x}{k_{O_2}}$$

or
$$(h_1 - h_0)\, k_{O_2} = \frac{y k_{O_2}}{k_{CO_2}} + x, \tag{4}$$

and
$$h_2 - h_0 = \frac{t_2}{t_1} \frac{x}{k_{O_2}}, \tag{5}$$

or
$$x = \frac{t_1}{t_2} k_{O_2} (h_2 - h_0);$$

substituting the value for x from equation (5) in equation (4)

$$y = k_{CO_2} \left[h_1 - h_0 - \left(\frac{t_1}{t_2} h_2 - h_0 \right) \right].$$

The value for y is obtained by absorbing the whole of the CO_2 expired during time t_1 and that evolved during the initial equilibration period. Hence to minimize errors, the equilibration time was made as short as possible (10–12 min.) and was included in t_2.

After most of the present experiments had been completed, Laser & Rothschild (1949) described a manometric apparatus for the measurement of respiration in the presence of CO_2. A number of additional experiments were therefore carried out using this method according to the details described in the paper of these authors.

Unless otherwise stated, all measurements were made at 37° C.

RESULTS

Before the respiration of *Gastrophilus* larvae could be measured by manometric methods, it was necessary to ascertain whether significant quantities of any volatile acid or base were liberated, especially since free ammonia is known to be excreted by other species of Dipterous larvae. The method adopted was to bubble air passed over a batch of respiring larvae into either N/100 acid or alkali. After some hours the acid or alkali (the effect of respiratory CO_2 had to be considered here) was titrated and the titre compared with a suitable control. These experiments produced no evidence for the formation by the larvae of any volatile substances which could be detected by this procedure, and hence it was concluded that manometric methods could safely be employed.

The O_2 *uptake of* Gastrophilus *larvae in the absence of* CO_2

The O_2 uptake of *Gastrophilus* larvae as measured over a period of hours by Warburg's direct method, i.e. by allowing 10–15 min. for temperature equilibration and absorption of CO_2 with KOH, showed that the rate of O_2 uptake declines in an irregular, but nevertheless continuous, manner. A typical series of values is shown in Table 1. This decline might be due to either or both of two causes, viz. acclimatization of the larvae to the experimental conditions imposed by the technique (i.e.

the so-called settling down effect well known to occur with insect material), or to the absence of carbon dioxide.

Table 1. *The* O_2 *uptake of two* Gastrophilus *larvae measured by Warburg's direct method. The larvae rested on moistened filter-paper*

Time in min.	Q'_{O_2}	Time in min.	Q'_{O_2}
15	−0·63	163	−0·28
30	−0·47	178	−0·20
45	−0·37	193	−0·25
60	−0·42	208	−0·19
75	−0·30	223	−0·22
90	−0·33	238	−0·17

There is little doubt that the former phenomenon is of considerable importance in the case of *Gastrophilus*; at 37° C. the larvae are very active and move about attempting to attach themselves to any material which they can pierce with their mandibles. However, of greater significance is the fact that after some hours in the manometer flasks larvae are occasionally found to be moribund. Such larvae can readily be distinguished by their red colour; this is due to the leakage of haemoglobin from the tracheal organ cells into the blood of the larvae, which is normally of a light amber colour.

From many experiments using the direct manometric method, it appeared very probable that this lytic effect and the progressive decrease in the Q'_{O_2} was due to absence of CO_2; Keilin in earlier unpublished experiments had obtained similar results which also led to this hypothesis, and his technique was adopted to obtain conclusive evidence.

A batch of larvae was divided into three groups; larvae of the first group were enclosed in a loose-fitting stainless steel cage, those of the second had their spiracular lips kept open by the insertion of a small brass paper fastener with sharpened ends bent outwards at *c.* 45°, and will be referred to below as larvae with opened spiracles, while in the third group the spiracular lips were ligatured, thus preventing any gas exchange through the spiracle. Attached to either the cage, the clip or the ligature thread, was a length of stiff wire the top end of which was fastened with a piece of split rubber pressure tubing inside the hollow neck of a Thunberg tube stopper. Thus, on inserting the stopper, the larvae were suspended in mid-air inside the Thunberg tube. The tubes were evacuated and filled with a variety of gases; except when carbon dioxide was a constituent of the gas phase, tubes were set up in duplicate, a filter-paper roll soaked with 2N–KOH being inserted near the bottom of one of the tubes. Larvae contained in the latter tubes, therefore, were in an atmosphere completely devoid of CO_2. A little water was placed inside the hollow stopper to prevent desiccation, the tubes were incubated at 38° and the larvae examined at hourly intervals. The results of three such series of experiments are shown in Table 2.

It will be seen that in normal larvae, or those with opened spiracles, leakage of haemoglobin from the tracheal cells into the blood occurred only in the absence of

CO_2; when the posterior spiracles were ligatured, thereby considerably reducing the loss of respiratory CO_2, the larvae remained normal. Results similar to those shown in Table 2 were obtained on a fourth experiment the duration of which was extended to 18 hr.

Table 2. *The effect of various gases in preventing the leakage of haemoglobin from tracheal organ cells* in vivo

Gas phase	1st hour		2nd hour		3rd hour	
	−KOH	+KOH	−KOH	+KOH	−KOH	+KOH
Air						
Spiracles normal	−	−	−	−	−	−
Spiracles opened	−	+	+−	+	+−	+
Spiracles ligatured	−	−	−	−	−	−
Evacuated						
Spiracles normal	−	−	−	−	−	−
Spiracles opened	+−	+	+−	+	+−	+
Spiracles ligatured	−	−	−	−	−	−
95% O_2+5% CO_2						
Spiracles normal	All Larvae Normal					
Spiracles opened						
Spiracles ligatured						
N_2						
Spiracles normal	−	−	−	−	−	−
Spiracles opened	+−	+	+−	+	+	+
Spiracles ligatured	−	−	−	−	−	−
CO_2						
Spiracles normal	All Larvae Normal					
Spiracles opened						
Spiracles ligatured						
O_2						
Spiracles normal	−	−	−	−	−	−
Spiracles opened	−	+−	−	+	−	+
Spiracles ligatured	−	−	−	−	−	−

+ =leakage of haemoglobin in all experiments, +− =leakage of haemoglobin either slight, or not observed in all three experiments, − =no leakage of haemoglobin into the blood.

In order to examine whether the absence of carbon dioxide affected the tracheal cells directly, the tracheal organ was dissected out and, suspended by the spiracular plate inside a Thunberg tube, was immersed in the insects' own haemolymph. In all other respects the experiment was the same as for whole larvae, except that the roll of KOH-moistened filter-paper was placed half way up the tube with a layer of grease immediately below to prevent any alkali reaching the blood or cells. The results were in agreement with the former series of experiments; in the absence of carbon dioxide haemoglobin leaked out from the cells into the surrounding blood, thus demonstrating that the cause of the impaired permeability of the tracheal cells is most probably loss of CO_2 from the haemolymph.

Evidence that a certain amount of CO_2 is indeed lost from the blood of larvae with lysed tracheal cells was obtained by pH measurement with the glass electrode. A sample of haemolymph taken from larvae with opened spiracles kept over KOH

in an atmosphere of N_2 had a pH of 7·14, which is 0·3–0·4 pH unit more alkaline than normal blood (cf. Levenbook, 1950*a*).

Measurement of the O_2 *uptake in the presence of* CO_2

The first series of experiments with the Haldane manometric method was carried out on 3rd instar *Calliphora erythrocephala* larvae at the stage when they had cleared their gut. The insects were obtained from a culture which had been in-bred in this laboratory for many generations. Fifty larvae were placed in the manometer vessel, 90 min. allowed for equilibration and the respiration measured at 25° C. To test whether carbon dioxide had any effect on the respiratory rate, the measurements were made in the presence or absence of KOH during each alternate hour as described above. The result of a typical experiment is shown in Table 3.

Table 3. *The respiration of* Calliphora *larvae in the presence and absence of* CO_2*

(Period of equilibration 90 min. Temperature 25° C.)

Time in hours	CO_2	Q'_{O_2}	R.Q.
1	Present	−0·94	0·75
2	Absent	−0·55	—
3	Present	−0·47	0·57
4	Absent	−0·52	—
5	Present	−0·50	0·66
6	Absent	−0·54	—
7	Present	−0·50	0·58
8	Absent	−0·49	—

* In these experiments the ammonia excreted by the larvae was not taken into account.

It will be seen that, apart from the atypical 1st hour's reading, the presence or absence of CO_2 was without effect on the respiration of *Calliphora* larvae. After the 1st hour the Q'_{O_2} values were reasonably steady, with an average value of 0·52. This is considerably lower than the corresponding figures obtained, among others, by Fraenkel & Herford (1938), Agrell (1947) and Hurst (1949), who found the Q'_{O_2} to be in the region of 1·0. This may be explained, at least in part, by the longer initial equilibration period in the present measurements. As evidence for this, another experiment may be cited in which the equilibration period was reduced to 45 min., when the Q'_{O_2} for the 1st hour in the presence of CO_2 was 0·92, and 0·87 during the 2nd hour in the absence of CO_2.

Measurement of the respiration of *Gastrophilus* larvae under similar conditions gave quite different results. A typical experiment using twenty fresh larvae is shown in Table 4.

The mean of eight series of experiments of this type have been combined in the histogram shown in Fig. 2. The average initial Q'_{O_2} of fresh larvae in the presence of CO_2 is close to 0·27, or about 0·13 ml. O_2 uptake per larva per hour at 37° C.

During each hour's respiration in the absence of CO_2 the Q'_{O_2} was lower than for a preceding or subsequent hour when CO_2 was present. However, the increased Q'_{O_2} during the subsequent hour as compared with the lower value in the absence of

CO_2 was insufficient to bring the rate of O_2 uptake to the pre-existing level before the CO_2 was removed. Hence, under the described experimental conditions, the Q'_{O_2} declined in a stepwise manner during 8 hr. The respiratory quotient (R.Q.) during the same period increased from the original value of *c.* 0·9 to 1·1–1·2. After some 8–10 hr. the final Q'_{O_2} was about 0·04–0·08, and once this low rate of O_2 uptake had been attained, removal of CO_2 from the atmosphere had only a very slight or even no effect on the respiration.

Table 4. *The respiration of fresh* Gastrophilus *larvae in the presence and absence of* CO_2

(Period of equilibration 90 min.)

Time in hours	CO_2	Q'_{O_2}	R.Q.
1	Present	−0·29	0·83
2	Absent	−0·25	—
3	Present	−0·26	0·86
4	Absent	−0·19	—
5	Present	−0·18	0·925
6	Absent	−0·11	—
7	Present	−0·14	1·09
8	Absent	−0·09	—

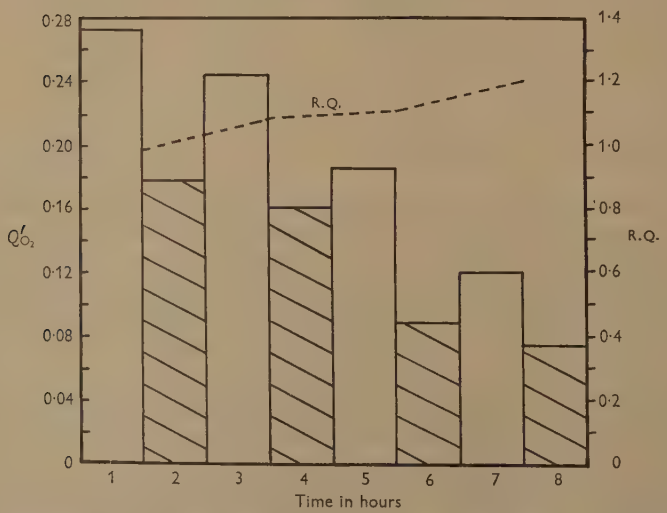

Fig. 2. Respiration of fresh *Gastrophilus* larvae. Period of equilibration in each of the eight experiments 90 min. Shaded columns denote O_2 uptake in the absence of CO_2. For further details see text.

A number of measurements were made on larvae which had remained in the manometer vessel at 37° C. for 18 hr. During this period either a very slow current of air was sucked through the apparatus, or KOH was present and all the taps were left open. It may be noted that the O_2 content of the closed manometer vessel was

sufficient to maintain the normal respiration rate of twenty larvae for 4–5 hr.; unless air was sucked through, it is uncertain to what extent gaseous diffusion through the taps was sufficient to supply the O_2 requirement of the larvae. However, *Gastrophilus* larvae are metabolically well adapted to survive anaerobically for considerable periods (Kemnitz, 1916; Dinulescu, 1932).

The experiments showed that the initial Q'_{O_2} of these larvae kept in the presence of CO_2 was only slightly lower than that of fresh larvae, ranging from 0·20 to 0·24, and the effect of removing CO_2 during each alternate hour was entirely similar to that described for fresh larvae. This is demonstrated by a typical experiment shown in Fig. 3.

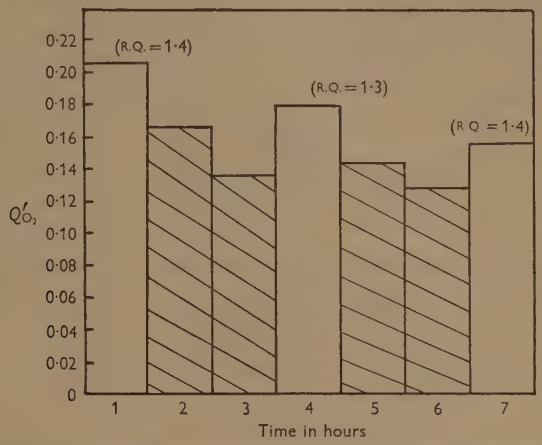

Fig. 3. O_2 uptake of twenty fresh *Gastrophilus* larvae after 18 hr. in the manometer vessel at 37° C. Shaded columns denote O_2 uptake in the absence of CO_2. For further details see text.

The Q'_{O_2} of the larvae equilibrated for 18 hr. in the absence of CO_2 was very low, of the order of 0·04, and not infrequently some of these larvae were found to be moribund with lysed tracheal cells. This low Q'_{O_2} was scarcely altered in the presence of CO_2 during the next 2 hr.

These latter experiments, therefore, would appear to show that the inhibitory effects on respiration ascribed to the absence of CO_2 can in no way be due to insufficient time allowed for settling down.

If the decline in the larval Q'_{O_2} is attributed to the absence of CO_2, it might be expected that the Q'_{O_2} would not diminish during a period of hours in the continuous presence of carbon dioxide; this was in fact found to be the case, as shown in Table 5.

The Q'_{O_2} values obtained by the Haldane manometric method were confirmed using the Laser-Rothschild (1949) apparatus; the results of one such experiment are shown in Table 6.

From the beginning of June-July, the diapause is broken and the larvae prepare to leave the alimentary tract and subsequently to pupate in the soil. This is correlated with a change in metabolism and, in particular, with an increase in the Q'_{O_2} (Table 7).

Table 5. *The respiratory rate of fresh* Gastrophilus *larvae in the presence of* CO_2

(Period of equilibration 90 min.)

Time in hours	Q'_{O_2}	R.Q.
1	−0·28	0·85
2	−0·24	0·89
3	−0·26	0·87
4	−0·25	0·90
5	−0·26	—

Table 6. *The respiratory rate of* Gastrophilus *larvae as measured in the Laser-Rothschild manometer*

(24-hr.-old larvae. Period of equilibration 30 min.)

Time in min.	Q'_{O_2}	Q'_{CO_2}	R.Q.
0–30	−0·28	0·24	0·85
30–60	−0·23	0·18	0·80
60–90	−0·23	0·22	0·93
Average for 90 min.	−0·25	0·21	0·86

Table 7. *The respiration of* Gastrophilus *towards the end of the 3rd larval instar*

(The figures refer to fresh batches of larvae equilibrated for 90 min.; measurements made by the Haldane manometric method.)

Date	Q'_{O_2}	R.Q.
16 June	−0·44	0·73
19 June	−0·455	0·76
24 June	−0·36	1·09
4 July	−0·39	1·04
15 July	−0·43	0·93

Table 8. *The respiration of* Gastrophilus *pupae*

(Haldane manometric method. Temperature 25° C.)

Time in hours	CO_2	Q'_{O_2}	R.Q.
1	Present	−0·22	0·79
2	Absent	−0·25	—
3	Present	−0·24	0·76
4	Absent	−0·23	—
5	Present	−0·23	0·80
6	Absent	−0·21	—

A similar increase in respiration is found in most insects on their emergence from diapause (see Wigglesworth, 1939). The larvae continue to be sensitive to the presence of CO_2, since all measurements made at this time showed that in its absence the Q'_{O_2} progressively declined as described above for diapausing larvae (cf. Fig. 2).

During June–July *Gastrophilus* pupae may be obtained in the laboratory by placing the larvae into a dish containing damp sawdust, when pupation occurs in 2–4 days at 24° C. In Table 8 data are presented for the respiration of twelve pupae 3 days

old; the Q'_{O_2} was found to be 0·23, and this was *not* affected by the absence of CO_2 from the gas phase; the latter finding was also confirmed using older pupae.

Experiments with single larvae

Normal larvae. To obtain an indication of the variation in O_2 uptake between different larvae, the modified Dickens & Šimer method was employed. The Q'_{O_2} during the first 3 hr. or so decreased with time since, as already mentioned, the equilibration period was insufficient to allow for the effect of settling down. Furthermore, once the solutions had been mixed so as to form the alkaline absorbing fluid, there was no means of re-introducing CO_2; hence determinations of the respiratory rate during the 2nd and 3rd hour were made by transferring larvae whose respiration had already been measured during the 1st hour as rapidly as possible to fresh manometer flasks previously warmed to 37° C.

The data obtained by this technique are presented in Table 9.

Table 9. *The respiration of single* Gastrophlius *larvae in the presence of* CO_2

	No. of experiments	Mean Q'_{O_2}	S.D.	Mean R.Q.	S.D.
Fresh larvae					
1st hour	19	−0·48	±0·1	0·72	±0·12
2nd hour	13	−0·31	±0·06	1·1	±0·2
3rd hour	3	−0·29	—	1·2	—
24 hr. larvae					
1st hour	18	−0·34	±0·07	0·81	±0·12
48 hr. larvae					
1st hour	5	−0·25	—	1·05	—

The Q'_{O_2} for fresh larvae during the 1st hour is considerably higher than the corresponding values found with the Haldane manometric apparatus. This may be attributed almost certainly to the much shorter equilibration period used for the single larvae. If the O_2 uptake after similar equilibration times be compared, i.e. during the 1st hour of the Haldane manometric method and the 3rd hour for the single larvae, the respective values −0·27 and 0·29 are in good agreement. It may also be seen that the variation in the Q'_{O_2} for the various larvae obtained from different batches is no greater than might be expected from such heterogeneous populations.

The effect of the spiracles on respiration

The respiratory function of the post-abdominal spiracle in *Gastrophilus* was investigated by measuring the respiration of a normal larva and comparing this value with that obtained using the same insect with either opened or ligatured spiracles.

Ligaturing the posterior spiracle decreased the Q'_{O_2} by about 95%, the values obtained ranging from 0·02 to 0·06. The carbon dioxide production of such larvae was not decreased to a similar extent, and consequently R.Q. values of 4–9 were obtained. These results are similar to those obtained by Fraenkel & Herford (1938) for *Calliphora* larvae with ligatured post-abdominal spiracles.

A ligature tied between the brain and the anterior spiracles, which are morphologically functional (Keilin, 1944), reduced the O_2 uptake in four experiments by 14–30%. However, it is improbable that this percentage represents the fraction of the total respiration passing through the anterior spiracles because of the following observations.

Larvae with ligatured posterior spiracles were suspended in Thunberg tubes and the latter evacuated, when the larvae became inflated to 2–3 times their normal size due to expansion of the enclosed air. After 18 hr. there was only a very slight diminution in volume, and the larvae, none of which died, returned to their normal dimensions when the vacuum was released. If gas were free to diffuse through the anterior spiracles, it seems unlikely that under the extreme conditions of these experiments the larvae would have remained in their inflated condition.

The O_2 uptake of larvae with opened spiracles was slightly, but consistently, increased. The average Q'_{O_2} for nine such larvae measured by the Dickens & Šimer method was increased by < 10% above that of normal larvae, while the R.Q. increased from 0·72 to 0·86.

The respiration of four larvae with opened spiracles was also measured in the Laser-Rothschild manometer; allowing an hour for equilibration, the following values were obtained for the subsequent 30 min. period: $Q'_{O_2} = -0{\cdot}37$, $Q'_{CO_2} = 0{\cdot}31$, R.Q. = 0·84. Comparing these figures with those for normal larvae (Table 6), it appears that opening the spiracles resulted in an increase of almost 50% in both O_2 uptake and CO_2 evolution. This increase in O_2 uptake is much larger than found for the corresponding value using individual larvae, and the explanation probably lies in the fact that in the latter case measurements were made during the 1st hour with only a short equilibration period; as already shown under these conditions the respiratory rate is higher than normal, and the spiracles would be more frequently, if not continuously, open to allow of the greater gas exchange. It might be expected, therefore, that forcing open the spiracles of such larvae would result in a relatively smaller increase in the Q'_{O_2}.

The influence of respiratory inhibitors

Some preliminary experiments were carried out employing respiratory inhibitors in order to assess the functional importance of the cytochrome system in the diapausing larvae.

Hydrogen cyanide. Cyanide is known to inhibit the respiration of almost all aerobic forms of life by combining in a reversible manner with heavy metal enzymes and, in particular, with the oxidized form of cytochrome oxidase.

By means of a fine hypodermic syringe with a micrometer-controlled plunger, known amounts of neutralized KCN were injected into *Gastrophilus* larvae through a ligature at the level of the 2nd segment. Controls were similarly injected with M/15 pH 6·8 phosphate buffer. The final hydrocyanic acid concentration in the larvae was calculated to vary from M/500 to M/1000. At these concentrations cyanide produced a rapid and profound decrease in respiration and killed the larvae.

For the more interesting results obtained by the use of gaseous cyanide, I am

indebted to Prof. D. Keilin for permission to quote some of his unpublished experiments. Briefly, these showed that, using the direct manometric method, gaseous cyanide inhibited the respiration of normal 3rd instar larvae almost completely, but the same concentration of cyanide inhibited to the extent of only 30–50% when the spiracles were forcibly opened. In a further series of experiments in which the larvae were allowed to respire in a closed vessel from which samples of air were withdrawn to determine the respiratory rate by actual gas analysis, the results were quite different. Under these conditions cyanide inhibited the respiration of normal larvae by about 30%, while with opened spiracles the inhibition was >75%. The difference in these results is almost certainly due to the presence or absence of carbon dioxide in the various experiments.

Sodium malonate. The particular interest attached to this respiratory inhibitor lies in the fact that Levenbook & Wang (1948) found a high concentration of succinic acid to be a normal constituent of *Gastrophilus* larva blood, and that the tracheal organ cells possess a very active succin-oxidase system. The question therefore arises as to whether the succinate in the blood is metabolized. Since malonate is a specific competitive inhibitor of the succin-oxidase system (Quastel & Wooldridge, 1928), any decrease in the respiration of larvae injected with this substance should be a measure of the O_2 uptake due to the oxidation of succinate.

10 μl. of M/5 sodium malonate were injected into the larvae by the method described above, the final concentration of malonate being approximately equal to the succinate already present. According to Keilin & Hartree (1949) this should be sufficient to inhibit almost completely the succin-oxidase system. Control larvae were injected with the same amount of phosphate buffer. The results of these experiments, one of which is shown in Table 10, indicated that injection of malonate did not inhibit respiration to a greater extent than did a similar concentration of phosphate buffer. This result was confirmed in the following manner.

Table 10. *The respiration of* 24 *hr.* Gastrophilus *larvae injected with either phosphate buffer or sodium malonate. Modified Dickens & Šimer method*

	Q'_{O_2}	Inhibition (%)	Q_{60_2}	Inhibition (%)	R.Q.
Control (2 larvae)					
Initial value	−0·34		0·26		0·77
After injection with phosphate buffer	−0·16	53	0·14	46	0·87
Experimental (3 larvae)					
Initial value	−0·39		0·35		0·90
After injection with sodium malonate	−0·21	54	0·17	52	0·82

Two lots of five larvae were placed on damp filter-paper in rubber-stoppered 50 ml. flasks kept at 37° C. Samples of air were then withdrawn from the flasks by piercing the stoppers with a hypodermic syringe moistened with salt solution, and the oxygen and carbon dioxide content of the samples determined by the Roughton

& Scholander (1943) technique. The larvae in one flask were then injected with phosphate buffer, the others with sodium malonate, after which the new respiratory rate was again similarly determined. The data so obtained are shown in Table 11. On account of the fact that no time was allowed for settling down, the Q'_{O_2} values shown in Table 11 are rather higher than would otherwise have been expected; nevertheless, it was again found that the injection of malonate did not inhibit the O_2 uptake as compared with the control larvae. It may be concluded, therefore, that *in vivo* the haemolymph succinate is not oxidized at any appreciable rate.

Table 11. *The respiration of fresh* Gastrophilus *larvae injected with either phosphate buffer or sodium malonate, as determined by gas analysis*

	Q'_{O_2}	Inhibition (%)	Q'_{CO_2}	Inhibition (%)	R.Q.
Control					
Initial value	−0·49		0·38		0·78
After injection with phosphate buffer	−0·32	35	0·31	18	1·0
Experimental					
Initial value	−0·49		0·42		0·86
After injection with sodium malonate	−0·34	30	0·32	24	0·97

Carbon monoxide and the effect of light. CO inhibits respiration due to its property of combining with the reduced form of heavy metals forming the prosthetic group of respiratory enzymes and carriers. Keilin & Wang (1946) found that the haemoglobin of the tracheal organ cells was peculiar in that it had a higher affinity for O_2 than for CO. On the assumption that the effect of CO could be demonstrated only when the O_2 tension was reduced to such an extent that any further reduction produced a significant decrease in the Q'_{O_2}, Keilin (unpublished) found by the direct manometric method that reducing the O_2 tension from 21 to 1% decreased the O_2 uptake by 50%, but even at this low tension 50% CO had no effect on the rate of O_2 uptake.

In extending Keilin's experiments, the effect of CO was examined in an atmosphere containing carbon dioxide, using the Laser-Rothschild manometer (Table 12).

Table 12. *The influence of* CO *on 24 hr.* Gastrophilus *larvae*

(Gas phase: 10% CO in air. Period of equilibration 35 min.)

Time in min.	Q'_{O_2}	Q'_{CO_2}	R.Q.
0–30	−0·098	0·184	1·87
30–60	−0·102	0·184	1·87
60–90	−0·100	0·194	1·71
Average for 90 min.	−0·099	0·187	1·81

The data show that a CO/O_2 ratio of 0·5 was sufficient to inhibit reversibly the O_2 uptake by some 60%. All the larvae were alive at the end of the experiment. Carbon

dioxide production was maintained at almost the normal rate, and the R.Q. approached 2. Higher values for the respiratory quotient, and inhibition of the Q'_{O_2} by some 80% were obtained by increasing the CO/O_2 ratio to 9; at this concentration the effect was still reversible and none of the larvae died.

Attempts were made to see whether the inhibition produced by CO was light-reversible; in the two experiments carried out the O_2 uptake did not increase on illumination, whereas CO_2 production was greatly stimulated. It was found subsequently that larvae when brightly illuminated showed an augmented CO_2 production even in the absence of CO (Table 13).

Table 13. *The effect of light on the respiration of* Gastrophilus *larvae*

(Laser-Rothschild apparatus. Period of equilibration 20 min. Gas phase air.)

Time in min.	Conditions	Q'_{O_2}	Q'_{CO_2}	R.Q.
0–30	Light	−0·23	0·34	1·45
30–60	Dark	−0·30	0·23	0·78
60–90	Light	−0·26	0·40	1·53

It was also noted that the illumination caused increased larval activity which, unexpectedly, was unaccompanied by a concomitant increase in Q'_{O_2}. The CO_2 production might therefore be due to aerobic 'physiological' glycolysis, i.e. the formation of gaseous CO_2 from the haemolymph bicarbonate (Levenbook, 1950*a*) by lactic acid liberated as a result of muscular activity.

DISCUSSION

The importance of CO_2 *in respiration*

In the extensive literature on insect respiration, Heller (1928) appears to be the only author to have reported that the respiration of an insect—the pupa of the moth *Deilephila euphorbiae*—was higher in the presence of carbon dioxide than in its absence. Unfortunately, no details were given. In the case of *Gastrophilus* the present experiments show that, to sustain the rate of O_2 uptake at its original level, the presence of CO_2 in the gas phase is essential for the 3rd instar larva, but is without effect on the respiration of the pupa. The explanation for this is apparently connected with the necessity for a high concentration of CO_2 in the larval haemolymph to maintain the tracheal organ cells (and possibly the cells of some or all of the other tissues) in a physiologically normal condition. The haemolymph CO_2 content is in turn a function of the CO_2 tension in the environment (Levenbook, 1950*a*); hence, when CO_2 is removed from the atmosphere, there is of necessity a concomitant loss of CO_2 from the blood and cellular damage results, which is manifested by lysis, a decline in the Q'_{O_2} and final death.

The fact that *Gastrophilus* pupae are not sensitive to CO_2 indicates a profound physiological change accompanying the transition to an aerobic, free-living mode of life. Levenbook (unpublished) has found that at the onset of pupation the CO_2 content of the haemolymph is greatly decreased, the intracellular tracheoles are withdrawn from inside the tracheal organ cells, and these show a marked increase

in viscosity and are less readily lysed. Superimposed on this are the normal changes, e.g. phagocytosis, which accompany metamorphosis, but the nature of the phenomenon occurring at pupation which renders this instar insensitive to CO_2 yet remains to be determined.

The reason why CO_2 should be required to maintain cellular impermeability is at present unknown; it cannot be due to a decrease in hydrogen-ion concentration, since the pH of the bright red blood of larvae with lysed tracheal cells was scarcely outside the range encountered in normal larvae. Jacobs (1922) showed that exposing a variety of organisms, e.g. *Paramecium*, *Colpidium*, *Arbacia* eggs etc., to an excess of CO_2 decreased protoplasmic viscosity after a short exposure, but increased the viscosity following a longer (1 hr.) period. A similar phenomenon affecting cell permeability may occur in *Gastrophilus*. It would be of interest to investigate the influence of different, known concentrations of carbon dioxide on lysis of the tracheal cells and respiration.

It has been shown for various insects (e.g. Hazelhoff, 1927; Stahn, 1928; Wigglesworth, 1935) that an increase in atmospheric CO_2 results in opening of the spiracles and hyperpnoea, while increasing the CO_2 tension beyond a certain threshold produces anaesthesia (Williams, 1946; Beadle & Beadle, 1949). This appears to be true only for species which are not adapted to high CO_2 tensions in their normal habitats. Thus, Kupka & Schaerffenberg (1947) found that several insect species (e.g. *Melolontha* spp., wireworm larvae) which live deep in the soil where the CO_2 concentration is high, were very resistant to gaseous CO_2 in which they could live for days, and showed little tendency to incur an O_2 debt; other species, which lived in the surface layers of the soil, reacted to higher CO_2 concentrations like the unadapted forms described above. The *Gastrophilus* larvae behave like the deep soil dwelling species in that they also survive for long periods in pure CO_2, and this gas does not induce either opening of the spiracles or anaesthesia. There can be little doubt, therefore, that the *Gastrophilus* larva is well adapted to the very high CO_2 tension in its environment (cf. Levenbook, 1950*a*).

The respiratory rate of *Gastrophilus* measured over short (10 min.) periods shows considerable fluctuation even in the presence of CO_2. This is in agreement with the observations of Punt (1943, 1950), who found that for those insects which possess a spiracular closing mechanism CO_2 was emitted at irregular intervals of 15–30 min., whereas *Calliphora* larvae which lack a closing mechanism gave off CO_2 continuously. It appears, although further work is necessary definitely to establish this fact, that *Gastrophilus* larvae can open the lips of their spiracular pouch and, by the play of muscular pressure transmitted through the haemolymph, fill the spacious tracheal trunks adjoining the spiracle with the external gas phase. The spiracular pouch is then closed, the oxygen in the enclosed air utilized and respiratory CO_2 given off, probably to a significant extent through the cuticle. This would account for the observations that when measured over short periods the respiratory quotient varies widely, frequently exceeding 1, and that larvae immersed in water will open their spiracles and give off bubbles of gas.

The function of the spiracles

The physiological function of the spiracles in respiration is not well defined by the present experiments. As regards the paired anterior spiracles, each of these is situated in a deep furrow and leads into a long and dense felt chamber (Keilin, 1944). It may be, therefore, that the reason why these spiracles in the inflated larvae placed in a vacuum were air-tight was due to compression of the felt chamber by the excessive internal pressure. If gas were unable to penetrate the felt chamber, very little or no gas could pass through the spiracle. Furthermore, the O_2 uptake of larvae with ligatured posterior spiracles was very low, yet their CO_2 output was still considerable. This indicates that CO_2 was lost either through the anterior spiracles, or the cuticle, or through both. Nevertheless, when such larvae were placed in the vacuum they apparently did not lose CO_2 since they remained inflated; this may be due either to the bulk of the dissolved tissue CO_2 having passed out through the cuticle at the time of evacuation, or perhaps to the molecular structure of the tightly stretched cuticle having become disorientated in such a way as to diminish its permeability to gases.

From the observation that (1) the respiration of inactive larvae with opened spiracles was considerably increased whereas under similar conditions that of active larvae was only very slightly increased, and (2) that very high tensions of CO_2 do not induce opening of the spiracular pouches in normal larvae, it seems probable that these are opened in response to O_2 lack and not to CO_2 excess.

The respiratory quotient

The wide fluctuation in the respiratory quotient calls for comment. The R.Q. is generally assumed to be an indication of the predominant substrate being metabolized (e.g. Richardson, 1929) but, as pointed out, among others, by Soskine & Levine (1946), the R.Q. of a whole animal cannot *per se* be regarded as sufficient evidence for the predominant type of foodstuff being 'burnt'.

The R.Q. in *Gastrophilus* is a combination of effects at physiological as well as biochemical levels. A range of experimental factors, which include removal of gaseous carbon dioxide, stimulation, the influence of bright illumination or various artificial treatments may, within a few hours, increase the R.Q. of fresh, diapausing larvae from the normal value of 0·85–0·9 to over 1; subsequently, by changing the conditions, values approaching the original may once more be attained. Such variation can scarcely be attributed to qualitative differences in metabolism, but to the effect of these factors on the permeability of the cuticle to CO_2, on the frequency of opening or closing of the spiracular pouch or the liberation of CO_2 from the blood and tissues due to a decrease in pH. In connexion with the last-mentioned phenomenon it has been found (Levenbook, 1950*a*, *b*) that bicarbonate was approximately equally distributed between the blood and tissues, and that the buffering capacity of the haemolymph was least at its normal pH. Furthermore, the blood lactic acid of larvae kept at 38° C. was much higher than of inactive larvae at 0° C. (Levenbook,

1950*c*). The conditions are therefore favourable for the formation by active larvae of gaseous CO_2 from bicarbonate which would yield high values for the 'respiratory' CO_2 and hence the R.Q. At present it has not proved possible to distinguish such inorganic CO_2 from true respiratory CO_2.

A genuine change in metabolism as shown by an increase in the 'metabolic' R.Q. does, however, occur: this is seen when the larvae emerge from diapause, or when they are kept *in vitro* for a number of days. Kemnitz (1916) has shown that under the latter conditions glycogen is partially converted to fat—a reaction for which the R.Q. is >1.

The action of inhibitors. In a detailed study of the effects of inhibitors on the eggs and embryos of the grasshopper *Melanoplus differentialis*, Bodine and co-workers (Bodine, 1934; Robbie, Boell & Bodine, 1938; Robbie, 1941) found that eggs in diapause were relatively cyanide insensitive compared with pre- or post-diapause eggs. Further, CO stimulated the respiration of diapausing eggs while inhibiting that of the developing egg (Bodine & Boell, 1934), and the latter inhibition was not light-reversible. It was concluded that the low respiration of eggs in diapause was not due to an enzyme system containing a heavy metal protein, whereas the higher respiratory rate during active development did involve a heavy metal protein, probably the cytochrome system.

The preliminary experiments reported in this paper indicate that, contrary to the diapausing *Melanoplus* egg, the respiration of the *Gastrophilus* larva during diapause does pass through a cyanide and CO sensitive heavy metal protein, probably, but not necessarily, cytochrome oxidase. The irreversibility of the CO inhibition by light may be due to the opacity of the larvae, since in a single experiment the respiration of a tracheal cell homogenate was found to be light-reversible.

There remains finally the interpretation of the lack of any inhibitory effects by sodium malonate. There is no doubt that *in vivo* the succinate in the blood is not oxidized to any appreciable extent by the succin-oxidase system of the tracheal organ cells, but *in vitro*, when the same cells are suspended in the insect's haemolymph, the succinate is rapidly metabolized.* However, even *in vitro*, malonate normally has hardly any effect, but there is a very pronounced inhibition when the cells are crushed. It is probable, therefore, that the deciding factor is the permeability of the tracheal cells to succinate and malonate. In this connexion, Keilin (unpublished) found that malonate injected into larvae with damaged tracheal cells (as evinced by lysis), did in fact inhibit respiration. This aspect of the work is being further investigated.

Due to its parasitic habitat, the physiology of the *Gastrophilus* larva bears certain resemblances to that of various helminths also parasitic in the mammalian alimentary tract. For the purpose of such a comparison, a discussion on the respiration of these parasites may be found in the works of Laser (1944), von Brand (1946), Hobson (1948) and Moulder (1950).

* The metabolism of the tracheal organ cells and the respiration of *Gastrophilus* pupae will be dealt with in subsequent papers.

SUMMARY

1. The respiration of the *Gastrophilus* larva has been investigated using three different manometric methods by which measurements may be made in the presence of carbon dioxide.

2. A new manometric apparatus of simple construction, designed for measurement of insect respiration in the presence or absence of CO_2, is described.

3. In the absence of CO_2 the respiration of *Gastrophilus* larvae progressively decreases and they eventually die. A manifestation of the CO_2 deficiency is an impaired cell permeability, resulting in leakage of haemoglobin from the tracheal cells into the blood.

4. The respiration of *Gastrophilus* pupae and *Calliphora* larvae was not affected by the absence of CO_2.

5. Both cyanide and carbon monoxide, but not sodium malonate, inhibited the respiration of *Gastrophilus* larvae during diapause.

6. The importance of allowing sufficient time for equilibration and settling down in the measurement of insect respiration is demonstrated.

I should like to thank Prof. D. Keilin, F.R.S., for his interest and advice in the present work. I am indebted to Dr H. Laser for carrying out all the experiments involving the use of the Laser-Rothschild manometer, and to the Agricultural Research Council for a maintenance grant.

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THE PHYSIOLOGY OF CONTRACTILE VACUOLES

VII. OSMOTIC RELATIONS IN A SUCTORIAN, WITH SPECIAL REFERENCE TO THE MECHANISM OF CONTROL OF VACUOLAR OUTPUT

By J. A. KITCHING, *From the Department of Zoology, University of Bristol*

(With Six Text-figures)

INTRODUCTION

In recent years two very different points of view as to the function and mechanism of contractile vacuoles have been developed. In peritrich ciliates the contractile vacuole appears to act as an osmoregulatory organ, controlling the body volume and internal osmotic pressure of the organism (Kitching, 1938, 1948); it is not known whether it also has an excretory function. On the other hand in amoeba, according to Hopkins (1946), the contractile vacuole performs excretion but not osmoregulation. Hopkins rejects my interpretation for peritrich ciliates, and by implication extends his own point of view to Protozoa generally. One of the purposes of this paper is to attempt to explain the discrepancy between these two very different interpretations of vacuolar activity. It is of course not entirely surprising that different experiments on different animals should have yielded different results.

In the hope of obtaining new evidence the present investigation was made on a class (or subclass) of Protozoa hitherto neglected in work on contractile vacuoles. The suctorian *Podophrya* has been found to be excellent material by reason of the ease of its culture, its attached habit, and its rotational symmetry. Its contractile vacuole has been found to respond in the expected way to changes in the osmotic pressure or temperature of the medium, and to increase greatly in rate of output when prey is captured and feeding is begun. This paper deals with osmotic relations and to some extent with the control of vacuolar activity.

MATERIAL

Podophrya sp., originating from a pond in the University grounds, was grown on silk threads in Petri dishes and deep culture dishes and fed on *Colpidium* sp. and *Paramecium caudatum*. The food organisms were grown in Bristol tap water fortified with a small quantity of a vegetable extract. This medium supported a considerable population of the necessary bacteria. To prepare a culture of *Podophrya* some of the food culture was placed in a dish, some silk threads were floated on the surface, and some *Podophrya* were added from an older culture. The *Podophrya* quickly devoured all the ciliates, and themselves produced motile forms, many of which attached themselves to the silk threads. The resulting *Podophrya* were at first opaque with granules of food material, but subsequently cleared. They were used for experiments 5–12 days after the culture had been set up. For a few experiments *Podophrya* was

cultured in 10% sea water, and was fed on *Colpidium* and *Paramecium* also cultured in 10% sea water; subsequent procedure was the same as for the tap-water cultures.

Podophrya is pear-shaped, and is attached (like a pear) by a stalk to the substrate. It carries a number of tentacles scattered over its surface. The body surface is covered by a pellicle, but this does not appear to extend over the tentacles. In some Suctoria, at any rate, it is known that the pellicle is perforated by many minute holes (Kormos, 1938). Except in the case of the experiment illustrated in Fig. 5, specimens of *Podophrya* having only one contractile vacuole were used. Sometimes two are present.

METHOD

The general methods were those used in earlier papers of this series (Kitching, 1948). The silk threads carrying the *Podophrya* were looped around the thermocouple wire, which was fixed to a glass plate on the stage of the microscope. The organisms were irrigated continuously throughout the experiments by the methods already described. The experiments were carried out at 15–20° C., but for any one experiment the fluctuations did not exceed $\pm 0{\cdot}5°$ C. The basic medium used in these experiments was Bristol tap water. All solutions were made up with this, and the organisms were irrigated with this for 1–2 hr. before observations were begun. Transfer from culture medium to tap water produced a slight transient increase or decrease in rate of vacuolar output. After the period of acclimatization, the *Podophrya* was observed first in tap water, then in the experimental solution, and finally in tap water again. In a few experiments there were further transfers to additional experimental solutions, always alternated with tap water.

RESULTS

Ethylene glycol

Eleven experiments were carried out. The concentrations ranged from 0·05 to 1·0 M. The results of four typical experiments are illustrated in Fig. 1. When *Podophrya* was transferred to ethylene glycol in 0·1 M concentration or over, the body shrank, the pellicle became wrinkled, the tentacles shortened in the case of the higher concentrations, and the contractile vacuole ceased activity. However, after 0·5–1·5 hr. the body filled out again to its original size, and the contractile vacuole resumed activity. When the *Podophrya* was returned to tap water the rate of output of the contractile vacuole at first increased considerably, but it subsequently fell off to about the level maintained at the beginning of the experiment. The tentacles temporarily became irregular or curly, but no significant changes in body volume were detected. With 0·05 M-ethylene glycol the results were similar though less extreme, and no detectable shrinkage occurred in that solution.

Sucrose

Nineteen experiments were carried out. The concentrations ranged from 0·005 to 0·1 M. A typical experiment, showing the usual range of variation in rate of vacuolar output, is illustrated in Fig. 2. The rate of vacuolar output was reduced in sucrose solution, and was restored to its original level or slightly over on return of the

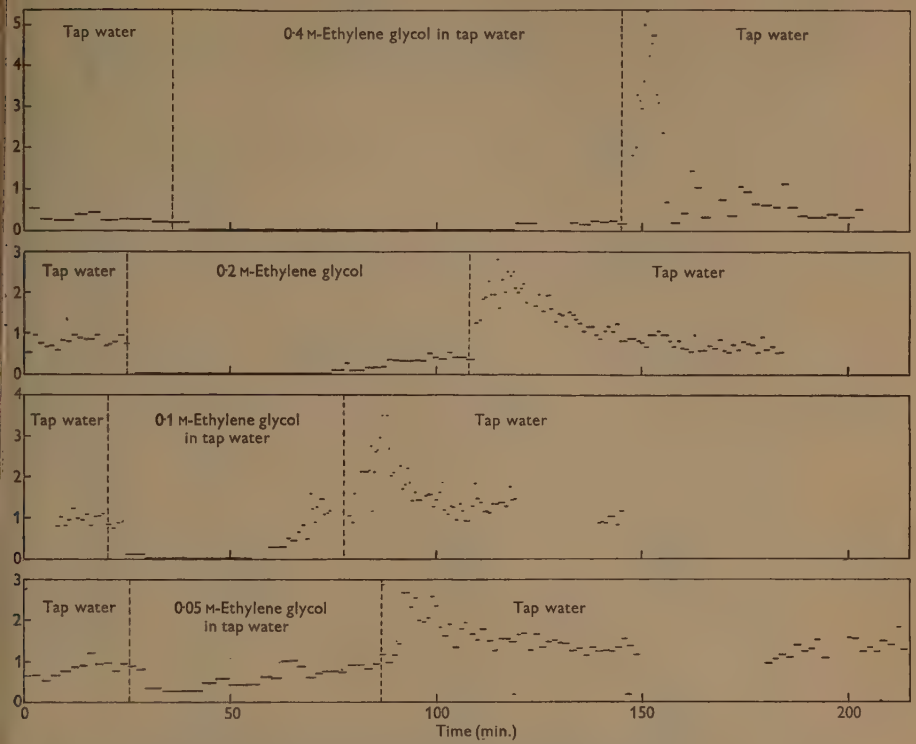

Fig. 1. The effect of treatment with a solution of ethylene glycol, and of return to Bristol tap water, on the rate of output of the contractile vacuole of *Podophrya*.

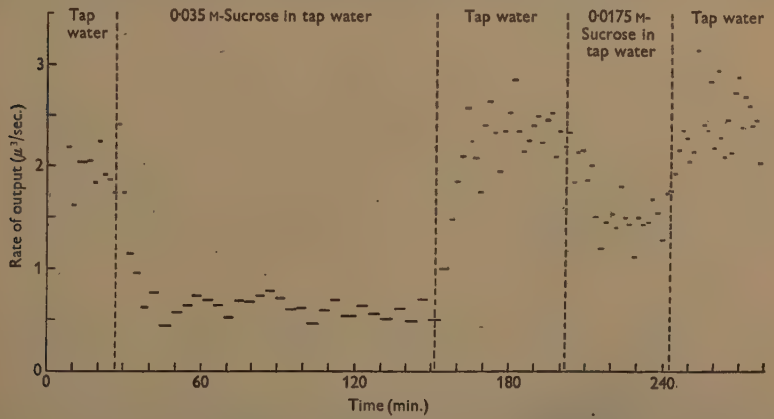

Fig. 2. The effect of treatment with a solution of sucrose, and of return to Bristol tap water, on the rate of output of the contractile vacuole of *Podophrya*.

Podophrya to tap water. In 0·05 M-sucrose or over no vacuolar activity could be detected. In all cases there was a slight lag in vacuolar response when the solution was changed (see p. 212). No change in body volume could be detected when *Podophrya* was placed in solutions of sucrose up to 0·04 M in concentration. A slight decrease occurred in 0·05 M-sucrose; in 0·07 M-sucrose the decrease was considerable; and in 0·1 M-sucrose the body became greatly shrunk, the pellicle was thrown

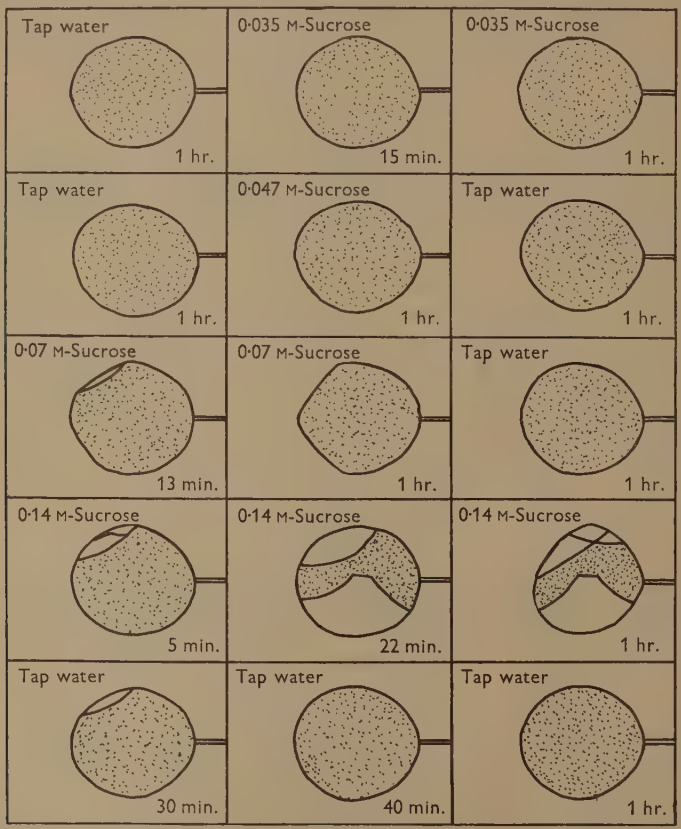

Fig. 3. The appearance in side view of a single *Podophrya* transferred alternately to tap water and increasing concentrations of sucrose for 1 hr. in each case. The tentacles are not shown.

into folds, and the tentacles became much shorter. Changes in body volume are illustrated most conveniently by a single experiment in which a *Podophrya* was transferred alternately to sucrose solution and tap water, the concentration of sucrose being increased each time; it remained for 1 hr. after each transfer. The appearance of this *Podophrya*, shown in Fig. 3, is typical of the results of all the experiments with sucrose.

The effect of sucrose on the rate of vacuolar output of *Podophrya* is summarized in Fig. 4 and Table 1. Similar results were also obtained in a few experiments with glycerol.

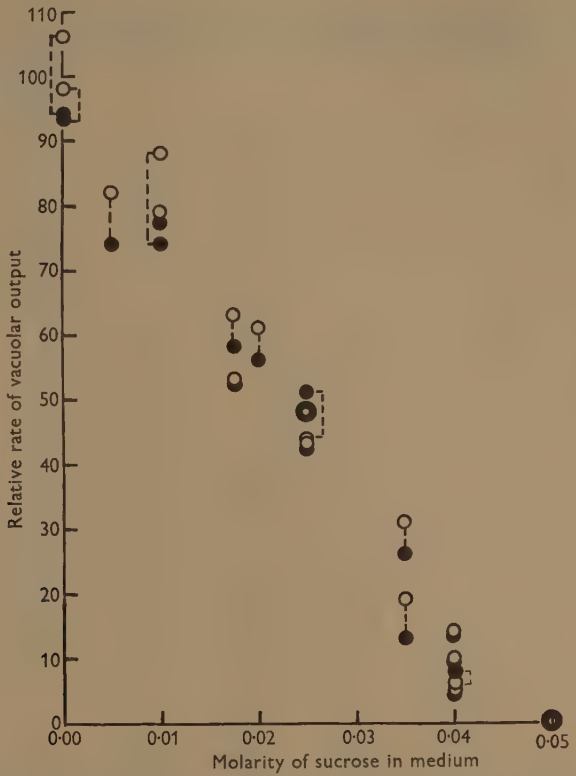

Fig. 4. The relation between the rate of output of the contractile vacuole of *Podophrya* and the concentration of sucrose in the medium. ○, the rate of vacuolar output in sucrose solution expressed as a percentage of the original rate in tap water; ●, the rate of vacuolar output in sucrose solution expressed as a percentage of the final rate in tap water. The two percentages calculated for each experiment are joined by a broken line where necessary. A thick circle indicates that the two percentages are the same. There are two coincident pairs at 0·05 M-sucrose.

Sea water

In four experiments a *Podophrya* cultured in 10% sea water was transferred to tap water. One of these is illustrated in Fig. 5. The rate of output of the contractile vacuoles increased greatly, but subsequently fell off to a level still considerably above that which had been maintained originally in 10% sea water (Table 2). The body volume was found to increase by about 5–10%. The significance of this change

Table 1. *Effect of sucrose in external medium on the rate of output of the contractile vacuole of the suctorian* Podophrya *sp. cultured in fresh water*

Exp. no.	Concentration of sucrose in medium (molarity)	Average rate of vacuolar output (μ^3/sec.)	No. of readings*	Duration of treatment† (min.)	Rate of vacuolar output in sucrose solution	
					(*a*) As percentage of initial rate in tap water	(*b*) As percentage of final rate in tap water
310150	—	1·25	11	25	—	—
	—	1·22	20	46	98	93
	—	1·31	15	29	—	—
090250	—	0·83	12	22	—	—
	—	0·88	20	33	106	94
	—	0·94	22	31	—	—
140250	—	0·87	13	31	—	—
	0·005	0·71	24	63	82	74
	—	0·96	18	27	—	—
161249	—	1·78	11	20	—	—
	0·01	1·57	30	71	88	74
	—	2·13	16	37	—	—
060350	—	0·95	10	20	—	—
	0·01	0·75	20	47	79	77
	—	0·96	15	32	—	—
081049	—	0·62	11	12	—	—
	0·025	0·30	9	23	48	48
	—	0·62	21	31	—	—
	0·025	0·27	21	38	44	51
	—	0·53	21	30	—	—
061049	—	1·45	8	13	—	—
	0·025	0·62	6	21	43	42
	—	1·48	9	15	—	—
011149	—	1·26	8	14	—	—
	0·035	0·24	2	28	19	13
	—	1·82	13	41	—	—
	0·0175	0·97	14	32	53	52
	—	1·88	18	30	—	—
141249	—	1·96	9	19	—	—
	0·035	0·60	26	62	31	26
	—	2·28	22	51	—	—
	0·0175	1·43	16	40	63	58
	—	2·48	23	36	—	—
121049	—	1·02	12	18	—	—
	0·04	0·05	9	45	5	4
	—	1·26	35	56	—	—
	0·02	0·77	21	37	61	56
	—	1·38	28	31	—	—
150250	—	0·61	20	32	—	—
	0·04	0·087	11	65	14	13
	—	0·68	12	34	—	—
160250	—	0·96	14	28	—	—
	0·04	0·094	5	68	9·8	9·5
	—	0·99	20	33	—	—

* This is the number of readings used in the estimation of the average rate of vacuolar output. It does not include the first few readings made after a change of medium and before a steady rate of output was attained.

† This is the whole period of treatment from the time of change of medium, and is not the value used for computing the average rate of output. In the case of the initial observation in tap water it does not include preliminary acclimatization to experimental conditions. No deductions have been made for occasional breaks in the continuity of observations.

Table 1 (*continued*)

Exp. no.	Concentration of sucrose in medium (molarity)	Average rate of vacuolar output (μ^3/sec.)	No. of readings*	Duration of treatment† (min.)	Rate of vacuolar output in sucrose solution	
					(*a*) As percentage of initial rate in tap water	(*b*) As percentage of final rate in tap water
170350	—	1·28	12	16	—	—
	0·04	0·075	7	61	6	8
	—	0·98	28	30	—	—
170250	—	0·57	8	22	—	—
	0·05	0·00	—	62	0	0
	—	0·67	24	42	—	—
070350	—	0·74	16	24	—	—
	0·05	0·00	—	32	0	0
	—	0·63	16	26	—	—
220250	—	0·73	8	19	—	—
	0·075	0·00	—	64	0	0
	—	0·87	16	62	—	—
200250	—	0·43	8	18	—	—
	0·075	0·00	—	31	0	0
	—	0·34	10	32	—	—
080350	—	0·99	7	17	—	—
	0·10	0·00	—	54	0	0
	—	1·04	31	78	—	—
090350	—	0·59	10	17	—	—
	0·10	0·00	—	60	0	0
	—	‡	—	54	—	—

‡ Variable, systole often incomplete.

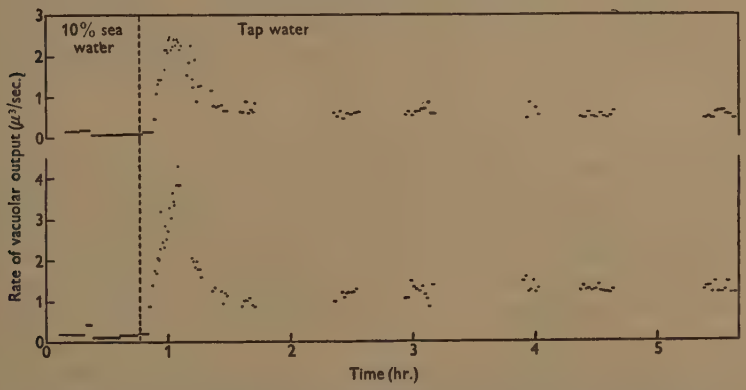

Fig. 5. The effect of treatment with tap water on the rate of output of the two contractile vacuoles of a *Podophrya* cultured in 10% sea water.

would have been doubtful were it not for the fact that in three of the four cases there was originally a kink in the body surface, and this quickly disappeared when the specimen was transferred to tap water. These kinks reappeared later as the vacuolar

output declined. When the *Podophrya* was first transferred to tap water the tentacles became irregular or curly, but they soon recovered their normal straight appearance. Similar results were obtained with a *Podophrya* cultured in 0·1 M-glycerol and transferred to tap water.

Table 2. *Summary of experiments in which* Podophrya *cultured in* 10% *sea water, was transferred to tap water*

Rate of vacuolar output in 10% sea water (μ^3/sec.)	Maximal rate of vacuolar output after transfer to tap water (μ^3/sec.)	Rate of vacuolar output at the end of experiment (μ^3/sec.)	Duration of treatment with tap water (min.)
0·15	2·90	0·63	165
0·14	3·00	0·94	345
0·76	22·6	2·54	422
0·28	6·10	1·80	350

DISCUSSION

Internal osmotic pressure of Podophrya

It may be inferred that the internal osmotic pressure of *Podophrya* under the experimental conditions was that of 0·04–0·05 M non-electrolyte. No shrinkage of the body could be detected in solutions of sucrose of 0·04 M concentration and below, whereas shrinkage could be detected in solutions of 0·05 M concentration upwards. The fact that shrinkage was very considerable in 0·1 M-sucrose shows that the quantity of osmotically inactive material was not sufficiently great to mask shrinkage when a sufficiently hypertonic medium was used; and the permanence of this shrinkage, together with a comparison of the effect on vacuolar activity of sucrose and glycerol on the one hand with that of ethylene glycol on the other, indicate that sucrose penetrates very poorly into *Podophrya*.

Osmoregulation of Podophrya

The relation between rate of vacuolar output and concentration of sucrose in the external medium is rectilinear (Fig. 4); this is what is to be expected for good osmoregulation. The experiments on *Podophrya* equilibrated with a solution of ethylene glycol, and then transferred to fresh water, constitute excellent evidence that the rate of output is increased in response to an increased difference of osmotic pressure between the body and the outside medium. Any change in body volume which may have occurred was normally too small to be detected ($< 10\%$). However, the fact that swelling does occur in wrinkled *Podophrya* when these are subjected to osmotic stress seems to suggest that swelling in other cases is prevented in part at least by the inextensibility of the pellicle by which the body is covered.

Comparison with amoeba

As a result of experiments on the minute fresh-water amoeba, *Amoeba lacerata*, which he cultured in various concentrations of sea water, Hopkins (1946) concluded

that the cytoplasm was practically isotonic with the outside medium, and that the rate of vacuolar output was inversely proportional to the concentration of sea water used. He inferred that the contractile vacuole contains dissolved excretory matter and grows by osmosis to a volume determined by the osmotic pressure of the cytoplasm. He also denied that contractile vacuoles perform osmoregulation either in amoeba or in Protozoa in general.

In spite of the importance of Hopkins's contribution, certain of his conclusions are open to criticism. He has not given any results for *A. lacerata* cultured in fresh water or in sea water more dilute than 5%, although it is in fresh water that osmoregulation is most likely to occur. Actually, owing to the difficulties of the material, a small excess of internal over external osmotic pressure, of an order which might reasonably be expected for an amoeba in fresh water, would not have been detected in his experiments. Moreover, he tested for osmoregulation by changing the external concentration of solutes which penetrate readily, and on amoebae fully adapted in respect of these solutes. No osmoregulatory response was to be expected under these conditions. Whether or not there occurs in amoebae in fresh water a difference of osmotic pressure sufficient to require a special osmoregulatory device, and whether that device is the contractile vacuole, has yet to be determined. Hopkins's conclusions may or may not prove correct for amoeba; they are not at present justified.

It would be useful to have more detailed information on the immediate change in rate of vacuolar output when *A. lacerata* is transferred to a lower concentration of sea water. The results given by Hopkins (1946, fig. 7) are rather variable, but they suggest an increase in vacuolar output to a level far above that shown by adapted amoebae. This would agree with the results obtained when *Podophrya*, cultured in 10% sea water, was transferred to fresh water (Fig. 5); the vacuolar output immediately increased greatly, but later fell off to a level still considerably above the original; salts no doubt leaked out, as in the case of *Amoeba lacerata*. On the other hand Hopkins's suggestion, that the osmotic work carried out by the contractile vacuole tends to remain constant, may become applicable after the excess of internal salts has leaked away, both in *A. lacerata* and in *Podophrya*.

It is now possible to suggest an explanation for the differences reported in various Protozoa in the relation between vacuolar output and osmotic pressure of the medium. For the fresh-water peritrich *Carchesium aselli* (Kitching, 1948) and the fresh-water suctorian *Podophrya*, the solute used (sucrose) penetrated very poorly, and the body volume did not alter measurably over the range of concentration under consideration. Therefore the response of the contractile vacuole was not seriously complicated by changes of the internal osmotic pressure of the organism, and may be regarded as purely under the influence of the osmotic stress imposed on the organism by the difference in osmotic pressure between the cytoplasm and the external medium. In the marine peritrichs, which were examined immediately after transfer to dilute sea water (Kitching, 1936), the body was swollen and the cytoplasm was therefore diluted; there was probably also some loss of salts. Nevertheless, the rate of vacuolar output rose more, in very dilute sea water, than could be accounted for on the assumption that the osmotic work remained constant. The curve

obtained, steeper at the lower concentrations than that given by Hopkins for *Amoeba lacerata* (Hopkins, 1946, p. 167), may perhaps be regarded as representing the combined influence of increased osmoregulation at the lower concentrations of the medium and decreased output (owing to the greater amount of osmotic work required for a given output) at the higher concentrations. In adapted *A. lacerata*, according to Hopkins, the osmotic concentration of the cytoplasm is the same as that of the outside medium, and only the 'osmotic work' factor operates.

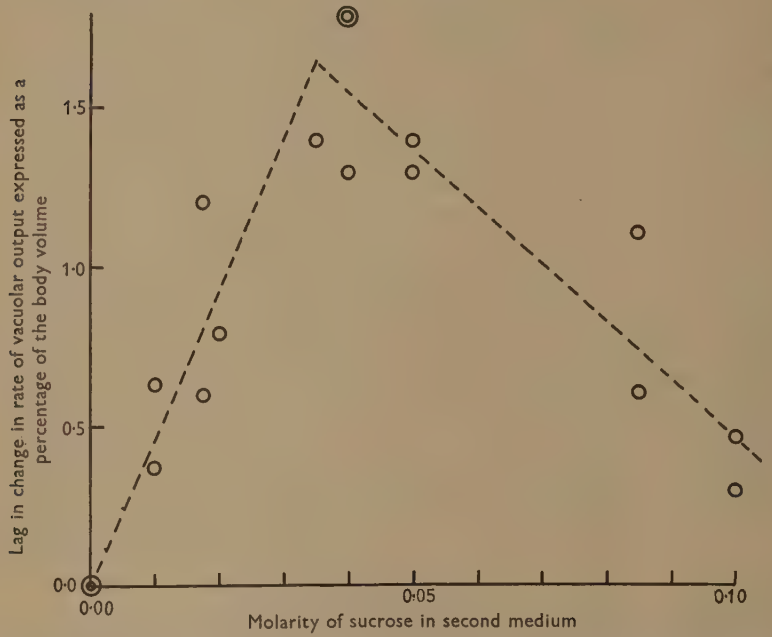

Fig. 6. The relation of the lag in change of rate of vacuolar output, expressed as a percentage of the body volume, and the concentration of sucrose to which *Podophrya* was transferred from tap water.

The regulation of vacuolar output

The automatic regulation of a condition, such as the temperature of a water-bath or the acidity of blood, is normally effected by small fluctuations in the condition itself, these operating the controlling mechanism (thermostat, respiratory centre, etc.). How then is the contractile vacuole of fresh-water Suctoria and peritrich ciliates operated accurately in accordance with the osmotic stress imposed on the organism? The contractile vacuole is presumed to prevent undue swelling and so to control the internal concentration of various dissolved substances. Change in rate of vacuolar output certainly follows a change in body volume in certain cases (Kitching, 1936, 1948); but no change in body volume was detected, nor at first sight would

any seem likely to have occurred, when a *Podophrya* was transferred to a solution of sucrose still hypotonic to the cytoplasm, as in the experiment illustrated in Fig. 2. However, we may plausibly infer that even in this case some small change of volume must result from the lag in vacuolar response. During the lag period the contractile vacuole was evacuating water at a rate greater than that finally attained in the sucrose solution. If we assume that the rate of uptake of water into the organism from the outside decreased abruptly with the transfer to sucrose solution, then a small decrease of body volume must have occurred during the lag. Conversely, the lag in response on transfer of the organism to a more dilute solution may be expected to result in a small increase in body volume. On reasonable assumptions the change in body volume may be estimated by the difference, from the time of change of medium up to the time when a new steady output is reached, between the total actual output and the total output which would have occurred had the new rate of output been established instantaneously. The results, for those experiments in which the rate of irrigation was sufficiently brisk and the records were complete over the transitional period, are plotted in Fig. 6 against the concentration of sucrose in the second medium (the first being tap water). It will be seen that the lag increases to a maximum of about 1½% of the body volume for 0·04 M-sucrose. For higher concentrations it falls off again; this is to be expected, since for concentrations hypertonic to the cytoplasm exosmosis of water through the body surface will accelerate the shrinkage of the body and so more quickly depress vacuolar activity. For the return from sucrose solution to tap water there was a corresponding lag of more variable magnitude; and this was much increased for concentrations of sucrose above about 0·04 M, again as would be expected as a result of the earlier exosmosis of water from the body.

Regulation of vacuolar output might conceivably be mediated in various ways; by the tension of the body surface, by the osmotic pressure in the cytoplasm or in some phase of it, by the cytoplasmic concentration of some particular solute or solutes, or by hydration or structural alteration of some cell component not necessarily in simple solution. The first mechanism is difficult to formulate and is inconsistent with certain unpublished observations; the second is inconsistent with various observations described in this paper or previously (Kitching, 1936), and the same is true of the concentrations of neutral salts in the organism. It is suggested that the control of vacuolar output may be attributed to the hydration of some protein or lipoprotein complex. The existence of a concentration of lipoid material around contractile vacuoles in many Protozoa, as indicated by blackening with osmic acid, may be not without significance in this connexion, in view of the observations of Schmitt & Palmer (1940) on the power of lipoid emulsions to contract and squeeze out water.

SUMMARY

1. Evidence from osmotic experiments indicates that the amount of osmotically inactive material in the suctorian *Podophrya* is small, and that the internal osmotic pressure of the cytoplasm is approximately that of a 0·04 M solution of non-electrolyte.

2. When the internal osmotic pressure of *Podophrya* is raised to an abnormally high level by equilibration with a solution of ethylene glycol or with dilute sea water, and the organism is then transferred to tap water, the rate of vacuolar output is temporarily raised far above its normal value. The body swells only slightly. This is taken as good evidence for osmoregulation.

3. When *Podophrya* is placed in a dilute solution of sucrose the rate of vacuolar output (relative to the original rate in tap water) decreases rectilinearly with the concentration of sucrose used, reaching zero at about 0·04 M. This is as would be required for good osmoregulation.

4. There is a slight lag in the response of the contractile vacuole to a change of medium. It is suggested that this delay in adjustment of the osmoregulatory mechanism must result in a slight change of body volume, which could be the basis for the control of vacuolar output.

I am glad to thank Prof. J. E. Harris for his continued support and advice during the course of this work. I am also greatly indebted to Mr Edward Livingstone, who developed the method of culturing *Podophrya* on silk threads and who has provided a steady supply of good cultures for this work.

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WAVE-RIDING DOLPHINS*

By A. H. WOODCOCK and A. F. McBRIDE†

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In an effort to explain the apparent ability of dolphins to ride the bow wave of a ship at a speed of 10 knots (Woodcock, 1948) it seemed necessary to show that the animals had weight while immersed. Measurements of the weight of an immersed *Stenella plagiodon* have been made.‡ First results indicate that a dead animal, which weighs 200 lb. in air, and is 6·5 ft. long, weighs 9·2 lb. when immersed in sea water. This animal, which was in good physical condition, was killed by injection just before the weighings were made.

Irving, Scholander & Grimel (1941, p. 152) have indicated that the lungs of dead *Tursiops truncatus* are collapsed. It is assumed therefore that the lungs of the dead *Stenella* were also collapsed at the time of weighing and that 9·2 lb. is near the maximum weight in sea water.

Irving *et al.* (1941) indicate that the volume of tidal air per 100 lb. of body weight for *Tursiops* is 2·7 l. Assuming the same proportions for *Stenella*, the tidal air volume for a 200 lb. animal is 5·4 l. At atmospheric pressure 5·4 l. of air will displace 5·54 kg. of sea water or 12·4 lb. Hence the *Stenella*, with tidal air expelled and weighing 9·2 lb., will weigh 9·2–12·4 lb., or −3·2 lb. in sea water with tidal air in its lungs. Thus these rough figures indicate that by breathing, dolphins can become lighter or heavier than the water which they displace. This indication is amply supported by the behaviour of *Stenella* in the tank at Marineland.

Stenella in the Marineland tank are observed to expel air from their lungs and to rest on the bottom for 4–6 min.§ Irving has said (personal communication) that dolphins can store oxygen in body fluids to such an extent that it is reasonable to suppose that they can stop breathing for several minutes with lungs empty of tidal air. It is therefore assumed that wave-riding dolphins remain 'heavy' in water by expelling tidal air.

Can a dolphin, which weighs 9·2 lb. in sea water fall down a wave front (either wind or bow wave) at a speed of 10 knots without swimming effort and at the same time gain sufficient dynamic lift to maintain position against gravity?

In order to remain at constant depth and position within the advancing face of a moving wave it seems clear that the dolphin must receive a lift of 9·2 lb. from the water and that his drag at wave speed must not exceed the component of gravity acting at the angle of inclination of the water layers. That is, D must not exceed

* Contribution No. 522 from the Woods Hole Oceanographic Institution.

† Former curator of Marine Studios, Marineland, Florida; died December 1949.

‡ These measurements were made by Dr Henry Kritzler, associate curator of Marine Studios, Marineland, Florida.

§ These observations were made by Dr H. Kritzler (l.c.) and W. E. Schevill of the Woods Hole Oceanographic Institution, Woods Hole, Mass.

$W-F_b$; where D is the total drag and $W-F_b$ is weight minus the buoyant force of the sea water. If an inclination of the wave of 15° is assumed,* the component of the gravitational force acting will be $W \sin 15°$ (i.e. $9{\cdot}2 \times 0{\cdot}259$, or about 2·4 lb.). A force of 3·1 lb. results when an angle of 20° is assumed.

A restatement of the above question now takes the following form: does the drag of a 6·5 ft. dolphin exceed 2·4 lb. when its speed is 10 knots and its lift is 9·2 lb.? Gray (1936) has given figures for computing the drag of a rigid body of the same size and shape as a dolphin, presumably a body having zero lift. Following Gray, and assuming a negligible increase in resistance due to a lift of 9 lb.,† the drag is derived as follows:

$$D=\frac{d\rho AV^2}{g}, \qquad (1)$$

where d = drag coefficient, ρ = density (64 lb. ft.$^{-3}$), A = surface area (15 ft.^{2}), V = speed (16·8 ft. sec.$^{-1}$), g = gravity (32 ft. sec.$^{-2}$), D = drag (lb.).

From the above equation and using Gray's tabulation of drag coefficients (see Table 1), it is found that the drag on a rigid body having the general size and shape of the *Stenella* would be 11·16 lb. when turbulent flow is assumed and 1·72 lb. when laminar flow is assumed (in sea water at a temperature of 70° F.).

Table 1. (*See Gray*, 1936.)

Reynolds' no.	Drag coefficient	
	Laminar flow	Turbulent flow
10^5	$2{\cdot}1 \times 10^{-3}$	—
2×10^5	$1{\cdot}5 \times 10^{-3}$	—
3×10^5	$1{\cdot}2 \times 10^{-3}$	—
4×10^5	$1{\cdot}0 \times 10^{-3}$	—
5×10^5	$0{\cdot}9 \times 10^{-3}$	$1{\cdot}0 \times 10^{-3}$
10^6	$0{\cdot}7 \times 10^{-3}$	$1{\cdot}5 \times 10^{-3}$
2×10^6	$0{\cdot}5 \times 10^{-3}$	$1{\cdot}6 \times 10^{-3}$
4×10^6	$0{\cdot}3 \times 10^{-3}$	$1{\cdot}56 \times 10^{-3}$
8×10^6	$0{\cdot}2 \times 10^{-3}$	$1{\cdot}4 \times 10^{-3}$
10^7	$0{\cdot}2 \times 10^{-3}$	$1{\cdot}3 \times 10^{-3}$
2×10^7	$0{\cdot}15 \times 10^{-3}$	$1{\cdot}25 \times 10^{-3}$

Reynolds' no. $= Vl/\nu$, where V = velocity, l = length and ν = kinematic viscosity of sea water.

Gray has indicated that the force required per lb. of muscle was excessive, if the resistance of an actively swimming dolphin is equal to that of a rigid model towed at the same speed. With the assumption of laminar flow, Gray found that the power developed per lb. of muscle agreed closely with that developed by other types of mammalian muscle.

If a reasonable balance of forces is to occur, it also seems necessary to assume laminar flow about the relatively motionless dolphin which is riding a bow wave. The

* W. H. Munk of Scripps Institution of Oceanography has said, in a personal communication, that 15° is a reasonable angle to assume for the inclination of the water surface on the advancing face of the larger wind waves. It would be interesting to know the slopes of bow waves of large sea-going vessels.

† Durand (1943, p. 132) indicates little change in drag of similar streamline bodies resulting from a small lifting force at low positive angles of attack.

total weight of the *Stenella* in sea water (9·2 lb.) is inadequate to balance a drag of 11·16 lb. This drag is about 4·6 times the resultant force of gravity acting at an inclination of 15° ($W-F_b$ sin 15°) and about 3·6 times this resultant at an angle of 20°. When the drag coefficient for laminar flow is used (see Table 1) the computed drag of 1·72 lb. is less than the available weight of 2·4 lb. Thus force exceeds drag when the speed is 10 knots and when the angle is 15°. For a 20° wave slope, force (3·1 lb.) equals drag at about 14·2 knots.

The above preliminary attempt to explain wave-riding of dolphins on the basis of the excess of total weight over buoyancy seems to give further support to the idea that the flow about their bodies is laminar.

Gray has suggested that, in contrast to rigid forms, dolphins may be able to prevent the onset of turbulence through effects arising from their swimming motions. Since wave-riding dolphins appear to be relatively motionless, the apparent necessity of assuming laminar flow about their bodies at 10 knots* implies that smooth flow may also occur with little or no swimming motion.

A further point, which arises from this study, concerns the significance of observations of the speed of dolphins about the bows of vessels or among wind waves on the open sea. If these animals can utilize gravitational force as a propelling aid on the inclined surfaces of bow or wind waves, then it seems clear that this force should be considered in any estimate of the dolphins' capacity for sustained work which is based upon observed speeds at sea. It is reasonable to suppose that dolphins may, by a burst of effort, place themselves in the bow wave of a passing vessel and then, with the aid of gravity, maintain this high speed with a swimming effort which is reduced in proportion to the force derived from the wave. Presumably the animals could equally well utilize, in a similar way, the energy of wind waves. For the wave-riding dolphin which is making no swimming effort the force of gravity is assumed to be equal and opposite to the drag force, thus making propulsive effort unnecessary.

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* This assumption is based upon Gray's table which shows turbulent flow beginning at a Reynolds' number of 5×10^5. More recent experimental work with carefully streamlined bodies has indicated, however, that in some rare cases laminar flow obtains at Reynolds' numbers as high as $1{\cdot}5 \times 10^7$ (see Hill, 1950, p. 216).

UNIT ACTIVITY IN THE MEDULLA OBLONGATA OF FISHES

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(With Plate 2 and one Text-figure)

Adrian & Buytendijk (1931) have recorded potential waves in respiratory rhythm from the isolated brainstem of the gold-fish. The waves could continue for 1 hr. or more.

During an investigation of the electrical activity of single cells in the central nervous system these results prompted us to explore with microelectrodes the medulla oblongata of fishes for unit activity in the respiratory centre.

Carp, varying in size from 15 to 30 cm. (nose to tail) have been used in all experiments. The actual size of the animal is, however, of little importance as the brain volume does not differ appreciably in bigger and smaller carps, the surplus space in the skull of the bigger specimen being occupied by a jelly-like substance.

The animals were anaesthetized by keeping them in a 1% urethane solution till the reflexes disappeared. They were then fixed to a splint by means of a bandage; a cannula was tied into the mouth to lead a current of tap-water through the gills. When required, 10% urethane solution was added. In later experiments the anaesthetized fish was placed in a basin with only the top of the skull above water-level, as the mouth-cannula was found to hinder the respiratory movements. Unhampered movement of the mouth is an essential part of the respiration especially in the bigger carps.

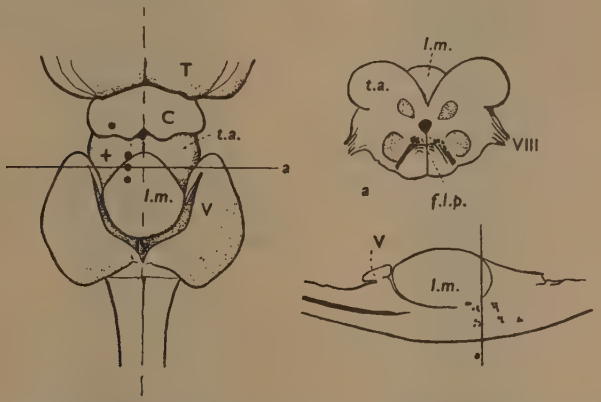
The skull was trepaned and firmly fixed by a special clamp. The brain was then freed from the embedding jelly and the cerebellum, which covers the bulb, was cut off at the insertion into the brain-stem. The medulla oblongata is thereby exposed as shown in Text-fig. 1 (left side), with the vagal lobes to right and left and the 4th ventricle, partly covered by the lobus facialis.

The electrical activity of the nervous tissue was studied by inserting bipolar microelectrodes, consisting of two enamelled platinum wires of 50μ gauge each, glued together and cut perpendicularly to their longitudinal axis. The electrodes are connected to the input of a condenser-coupled push-pull amplifier, whose output is led to a Philips cathode-ray oscillograph, type GM 3156. A power amplifier and loudspeaker are connected for acoustic control of the action sounds. The apparatus is fully described by Woldring (1950) and Dirken & Woldring (1950).

The electrodes were inserted by means of a Zeiss-micromanipulator, specially adapted to our purpose. The bulb was searched for unit potentials along antero-posterior and transverse lines 0·5 mm. apart, the crossing of the commissura infima

and the mid-line, caudal to the tip of the 4th ventricle, serving as a point of reference in the various experiments. The respiratory potentials have been recorded photographically, together with the movements of the gills which were connected to a lever carrying a small mirror.

Most of the unit activity has been found in the central part of the medulla oblongata, whereas large parts of the vagal and facial lobes have proved to be almost free of electrical activity. Volleys of spike potentials in respiratory rhythm can be detected in two small strips to the right and the left of the mid-line at the frontal border of the facial lobe just behind the acoustic tubercles. Pl. 2*a* shows the discharges of a single respiratory unit picked up in the medulla oblongata 0·5 mm. anterior to the frontal border of the facial lobe, 0·5 mm. to the right of the mid-line and 1·25 mm. below the dorsal surface.



Text-fig. 1. Brain-stem of the carp after removal of the cerebellum (*c*). *v.*, lobus vagus; *l.m.*, lobus impar; *t.a.*, acoustic tubercle; IV, fourth ventricle; *f.l.p.*, fasciculus long post.; black dots, respiratory activity; + electrical activity on acoustic stimulus.

In the record upward deflexion of the curve corresponds to inward movement of the gills; the record shows that bulbar activity is associated with inward movement of the gills. The frequency of the discharge is highest at maximal adduction and decreases during abduction of the gills. With maximal abduction discharges are absent.

Pl. 2*b* represents the discharge pattern 0·25 cm. ventral to the spot where the former record was taken. Here several units contribute to the volley, which shows the same general behaviour as the single unit activity of Pl. 2*a*.

The structures giving rise to these discharges were partly covered by the lobus facialis and were found 2 mm. below the dorsal surface. Those picked up anterior to this lobe were met at a depth of 1–1·5 mm. The region of respiratory activity lies *c.* 0·5 mm. from the mid-line and has an extension of 1–1·5 mm. in the antero-posterior direction. In one case a further respiratory volley was picked up from

a spot anterior to the left acoustic tubercle under the attachment of the cerebellum (dots in Text-fig. 1).

In the acoustic tubercles, about 1–2 mm. from the mid-line (Text-fig. 1), a volley of spike potentials was elicited by acoustic stimulation. Even whispering at a distance of 3 m. proved to be effective.

On examining the microscopic sections several groups of rather large cells were found in the two strips near the mid-line where nearly all respiratory volleys had been detected. These cells lie ventro-lateral to the fasciculus longitudinalis posterior and partly give rise to outgoing fibres (see Text-fig. 1, right side, representing longitudinal and transverse sections of this particular region). As the needle tracks were not discernible on account of the softness of the tissue, it was not possible to verify exactly the position of the electrodes. The depth to which the electrodes were inserted, however, agrees with that of these large cells. Presumably we have recorded the discharges of the neurons of the cranial motor nerves VII, IX and X, whose nuclei lie in a row parallel to the fasciculus longitudinalis posterior according to Kappers, Huber & Crosby (1936).

The segmental arrangement of these nuclei innervating the gill muscles has been emphasized by Hyde (1904).

The lobi vagi and the lobus facialis did not exhibit much electrical activity; spike potentials were rather scarce here. This is readily understood in view of the sensory function of these out-growths which belong to the gustatory system (Kappers, *et al.* 1936). No relation with respiratory movements was found.

SUMMARY

In the medulla oblongata of carp respiratory potentials were found in two small strips 0·5 mm. to left and right of the mid-line at the level of the caudal border of the acoustic tubercles and at a depth of about 2 mm. below the dorsal surface. Acoustic potentials were discovered in the acoustic tubercles. No particular unit activity was found in the vagal and facial lobes.

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EXPLANATION OF PLATE 2

Records of the electrical activity of respiratory cells in the medulla oblongata of the carp. Registration of gill-movements: adductions of the gills is represented by an upward movement of the signal-line. Time signal: $\frac{1}{5}$ sec. *a*, single unit, discharging in respiratory rhythm. *b*. respiratory volley of several units. For further details see text.

WOLDRING AND DIRKEN—UNIT ACTIVITY IN THE MEDULLA OBLONGATA OF FISHES

AERODYNAMICS OF FLAPPING FLIGHT WITH APPLICATION TO INSECTS

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(With Fifteen Text-figures)

INTRODUCTION

The problem of natural flight is one of long-standing interest, both intrinsically and in connexion with mechanical flight. Rayleigh (1883) was perhaps the first to give a satisfactory explanation of the soaring flight of birds, and Walker (1927) gave a satisfactory quantitative discussion of the flapping flight of birds in a particular case. Hoff (1919), using the data of Demoll (1918), attempted to bring the flight of insects into the domain of conventional aerodynamics by showing that lift coefficients of reasonable value were required. He used only the linear velocity of flight to evaluate these coefficients and ignored the motion of the wings. He also pointed out the similarity between the flow pattern around an insect and around a propeller or actuator disk, a similarity which must hold from momentum considerations, independently of the action of the wings (Bairstow, 1939). Demoll (1919) indicated several errors and omissions in Hoff's work, so that in a number of cases the lift coefficients required were inordinately high. Finally, a most thorough experimental investigation was carried out by Magnan (1934), who was, however, unable to explain his results theoretically.

The difficulty of high lift coefficients has not been removed by other investigations which have indicated lift coefficients larger than those normally expected in wind-tunnel measurements. It has, therefore, been believed that insects must utilize some special mechanism in flight not present in conventional aerodynamic phenomena.

In this paper the mechanism of insect flight will be examined in detail in order to find some possible explanations for the high lift coefficients, as well as for other characteristic features of insect flight. General expressions will be obtained for the force acting on a wing surface element moving in an arbitrary manner. This force will be resolved into lift and drag components with respect to the relative wind, and integrated over the surface of the wing and averaged over the period of a wing beat in order to determine the total average vertical and horizontal forces exerted by the insect. The former is the required vertical force (weight of insect), the latter the required thrust (drag of insect's body). Average values of the lift and drag coefficients of the wings, $\bar{C}_L$ and $\bar{C}_D$, can then be determined, since there are two known forces given, and two unknown coefficients to be determined.

The scalar product of the vector force on the wing element into the vector velocity of the element with respect to the insect, integrated and averaged as before, gives the mechanical power exerted by the insect in flight.

The absolute magnitude of the total force on the wing element, integrated and averaged, will give a lower bound to the force coefficient for the average total force, $(\bar{C}_L^2+\bar{C}_D^2)^{\frac{1}{2}}_{\min.}$.

The power, the lift, drag and total force coefficients computed for the twenty-five insects for which data is available, will, when plotted against the other parameters of insect flight such as mass and ratio of flapping to linear velocity, reveal a number of interesting and systematic characteristics of insect flight. The flight data, summarized in Table 1 (p.), were taken from the work of Magnan, and were supplemented by measurements on specimens of the U.S. National Museum.

For those who wish to draw conclusions from this work without verifying the mathematical details, it may be said that the principal mathematical problem is to justify replacing the instantaneous velocities by suitably chosen averages over position and time. Once this process is admitted practically all of the qualitative conclusions may be reached by drawing vector diagrams similar to those of Fig. 4, with these average velocities for any particular case. In fact, if the averaging precepts are admitted, quantitative conclusions can be reached by drawing to scale, and with all small components such as v and w included, diagrams like Fig. 4.

DERIVATION OF THE FUNDAMENTAL FORMULAE

It is assumed that the lift force $\mathbf{dF}_s$ on an element of wing surface $c(r)dr$ is given by

$$\mathbf{dF}_s=(\tfrac{1}{2})\rho C_L c(r)\,dr\,|\,\mathbf{l}\times\mathbf{W}\,|^2\{(\mathbf{l}\times\mathbf{W})/|\,\mathbf{l}\times\mathbf{W}\,|\}, \tag{1}$$

and the drag force is given by

$$\mathbf{dF}_p=-(\tfrac{1}{2})\rho C_D c(r)\,dr\,|\,\mathbf{l}\times\mathbf{W}\,|^2\{(\mathbf{W}-\mathbf{W}.\mathbf{l}\mathbf{l})/|\,\mathbf{W}-\mathbf{W}.\mathbf{l}\mathbf{l}\,|\}. \tag{2}$$

The quantities which appear in these two formulae are defined as follows (see Fig. 1). Vectors are denoted by bold face, their scalar magnitude by two vertical lines.

$\mathbf{l}$, $\mathbf{m}$, $\mathbf{n}$ are mutually perpendicular unit vectors parallel to the axis of the wing, perpendicular to the axis of the wing in the instantaneous plane of beating, and perpendicular to this plane, respectively. The plane of beating is not the plane of the wing, but the plane in which its axis is moving. ρ is the air density. W is the relative wind or velocity of the surface element of the (right) wing with respect to the air, and is given by

$$\mathbf{W}=(\mathbf{n}\Omega\times\mathbf{l}r)-\mathbf{U}. \tag{3}$$

Ω is the instantaneous angular velocity of the wing element, and $\mathbf{U}=(0,\,-(V+v),\,-w)$ is the velocity of the air with respect to the insect's body. V is the velocity of flight and v, w are the induced velocities. $c(r)$ is the chord. r and t are the independent variables – distance along the wing and time. In the general case all of the quantities appearing in eqs. (1) and (2) (except ρ and $c(r)$) are functions of r and t. If the wing is assumed not to twist or bend, the unit vectors and Ω will be independent of r. C_D evidently represents the profile drag coefficient, since the relative wind includes the induced velocities.

Average values for the induced velocities v, w, or velocity increments of the slipstream, are given from momentum theory (Durand, 1935). They are independent of the mechanism of the wing action, and are obtained in first approximation by

requiring that v and w at the insect have one-half the final value necessary to provide the necessary vertical force and horizontal thrust:

$$\text{Lift (vertical force):}\quad L = Mg = \pi R^2 (V^2 + w^2)^{\frac{1}{2}}\, 2w, \tag{4}$$

$$\text{Thrust:}\quad T = (\tfrac{1}{2})\, C_{Db}\, S_b \rho V^2 = \pi R^2 (V^2 + w^2)^{\frac{1}{2}}\, 2v. \tag{5}$$

R is the length of the wing, M is the mass of the insect, g is gravity, and S_b the body cross-section area and C_{Db} the body drag coefficient. Since, by division of the above,

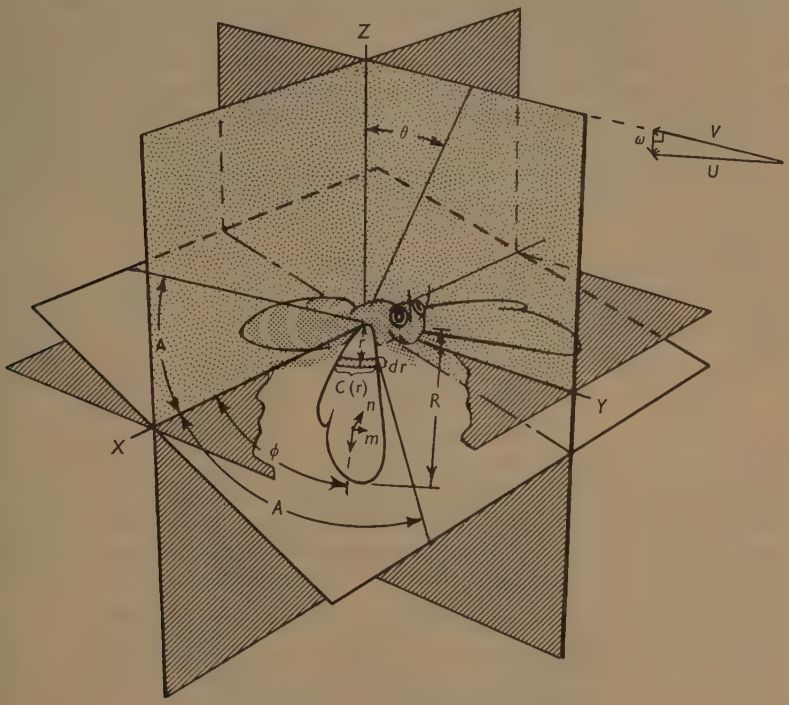

Fig. 1. Quantities defining motion of insect in flight.

$v/w = T/L$, and for the insects considered the thrust T is small compared to the vertical force L. v is small compared to w, and will be neglected, since w turns out to be small compared to the other velocities involved. The solution for w is given by

$$w^2 = V^2 \tfrac{1}{2}[-1 + (1 + L^2/\pi^2 \rho^2 R^4 V^4)^{\frac{1}{2}}]. \tag{6}$$

Eqs. (1) and (2) express the assumption that the determining velocity (squared) for both lift and drag is the component of the relative wind perpendicular to the axis of the wing, which is $|\mathbf{l} \times \mathbf{W}| = |\mathbf{W} - \mathbf{W}.\mathbf{ll}|$. The subscripts s and p indicate perpendicular (senkrecht) and parallel *to that component*, $\mathbf{W} - \mathbf{W}.\mathbf{ll}$. The last factor in braces gives the direction cosines of the forces. The drag is assumed to be perpendicular to the axis of the wing, hence the subtraction of the $\mathbf{l}$ component of $\mathbf{W}$, $\mathbf{W}.\mathbf{ll}$ in the

direction cosine. Forces along the length, or axis, of the wing tend to cancel for lift and thrust on the upper and lower half of a wing beat and overall between the right and left wing, since they are in opposite directions for opposite wings. Also they do not contribute to the power (eq. (8)) since they are perpendicular to the displacement of the wing elements, referred to the insect.

The assumption that the force is proportional to the square of the relative wind component perpendicular to the axis of the wing is the conventional one in helicopter and sideslip performance calculations, and applies primarily to steady flow. Steady flow is far from realized in flapping flight, unless the variable or flapping components of velocity are small compared to the linear velocities, or the flapping velocity changes by a small fraction of itself over a displacement equal to the chord. The assumption of the steady state force formula is made primarily so that the computed results for C_L and C_D can be compared with conventional aerodynamic data, as will become apparent from the subsequent discussion. However, there is indirect evidence given below that this assumption is justified, at least in part, for the problem under consideration.

The total force on the surface element of the wing is

$$\begin{aligned} |\,\mathbf{dF}\,| &= (|\,\mathbf{dF}_s\,|^2 + |\,\mathbf{dF}_p\,|^2)^{\frac{1}{2}} \\ &= (\tfrac{1}{2})(C_L^2 + C_D^2)^{\frac{1}{2}} \rho c(r)\, dr\, |\,\mathbf{l}\times\mathbf{W}\,|^2. \end{aligned} \tag{7}$$

The power expended in exerting the forces dF_s and dF_p is

$$\begin{aligned} dP &= (\mathbf{dF}_s + \mathbf{dF}_p).(\mathbf{n}\Omega\times r\mathbf{l}) \\ &= dP_{F_s} + dP_{F_p}, \end{aligned} \tag{8}$$

where P_{F_s} and P_{F_p} are the power contributions due to C_L and C_D forces.

The above formulae are general; the method of integrating and averaging them would depend on the particular example to which they were applied. In case the normal to the instantaneous plane of beating is the same for both wings, and remains in the yz plane, as in Fig. 1, the unit vectors $\mathbf{l}$, $\mathbf{m}$, $\mathbf{n}$ for the right wing are, in the x, y, z system,

$$\left.\begin{aligned} \mathbf{l} &= (\cos\phi,\ \sin\phi\cos\theta,\ -\sin\phi\sin\theta), \\ \mathbf{m} &= (-\sin\phi,\ \cos\phi\cos\theta,\ -\cos\phi\sin\theta), \\ \mathbf{n} &= (0,\ \sin\theta,\ \cos\theta). \end{aligned}\right\} \tag{9}$$

If θ, the inclination to the vertical of the normal to the instantaneous plane of beating (Fig. 1), is a constant, i.e. the wings beat in a plane, then $\Omega = d\phi/dt$. With these two specializations, eqs. (1) and (2) give (bars indicate average)

$$\begin{aligned} \overline{\mathbf{F}}_s = (\overline{X}_s, \overline{Y}_s, \overline{Z}_s) &= \nu \iint_{r,t} \mathbf{dF}_s(r,t)\, dr\, dt \\ &= \nu \iint_{r,t} (\tfrac{1}{2})\rho C_L\, 2c(r)\,(W_m^2 + W_n^2)^{\frac{1}{2}}\, dr\, dt \\ &\quad \times (w\sin\phi\cos\theta + V\sin\phi\sin\theta, \\ &\quad\ r(d\phi/dt)\sin\theta - w\cos\phi, \\ &\quad\ V\cos\phi + r(d\phi/dt)\cos\theta) \end{aligned} \tag{10}$$

$$\overline{\mathbf{F}}_p=(\overline{X}_p,\overline{Y}_p,\overline{Z}_p)=\nu\iint_{r,t}\mathbf{dF}_p(r,t)\,dr,dt$$

$$=-\nu\iint_{r,t}(\tfrac{1}{2})\rho C_D 2c(r)\,(W_m^2+W_n^2)^{\frac{1}{2}}\,dr\,dt$$

$$\times(-r(d\phi/dt)\sin\phi-V\sin\phi\cos\phi\cos\theta+w\sin\phi\sin\theta,$$

$$+r(d\phi/dt)\cos\phi\cos\theta+V\sin^2\theta+V\cos^2\phi\cos^2\theta+w\sin^2\phi\sin\theta\cos\theta,$$

$$-r(d\phi/dt)\cos\phi\sin\theta+w\cos^2\theta+w\cos^2\phi\sin^2\theta+V\sin^2\phi\sin\theta\cos\theta). \qquad (11)$$

The range of integration over r is the length of the wing; 0 to R, and in t over the period $1/\nu$ of a wing beat, ν being the frequency of flapping.

In eqs. (10) and (11)

$$\left.\begin{aligned} W_m^2+W_n^2&\equiv|\,\mathbf{l}\times\mathbf{W}\,|^2\equiv|\,\mathbf{W}-\mathbf{W}.\mathbf{ll}\,|^2\equiv|\,\mathbf{W}.\mathbf{mm}+\mathbf{W}.\mathbf{nn}\,|^2,\\ W_m&=r(d\phi/dt)+(V\cos\theta-w\sin\theta)\cos\phi,\\ W_n&=V\sin\theta+w\cos\theta,\end{aligned}\right\} \qquad (12)$$

and the denominators of the direction cosines in eqs. (1) and (2) have been cancelled against the velocity-squared factor. The factor 2 before $c(r)$ takes account of left plus right wings.

The total average power* is from eq. (8)

$$\overline{P}=\{w\overline{Z}_s+V\overline{Y}_s\}+\nu\iint_{r,t}\mathbf{dF}_p(r,t).(\mathbf{n}(d\phi/dt)\times r\mathbf{l})\,dr\,dt$$

$$=\{w\overline{Z}_s+V\overline{Y}_s\}+\nu\iint_{r,t}(\tfrac{1}{2})\rho C_D\,2c(r)\,dr\,dt\,(W_m^2+W_n^2)^{\frac{1}{2}}\,r\,d\phi/dt$$

$$\times[r(d\phi/dt)+(V\cos\theta-w\sin\theta)\cos\phi]. \qquad (13)$$

Here the first term in the braces is the exact expression for P_{Fs}. The expression for power on the basis of the momentum theory (using eqs. (4) and (5)), is $VT+wL$, which is the rate of change in kinetic energy in the slipstream on passing the insect required in order to provide the net thrust $T=(\tfrac{1}{2})\rho C_{Db}S_bV^2$ and lift $L=Mg$. Thus when $C_D=0$ ('ideal' fluid) the power determined from momentum theory and the power determined by integrating force × velocity (i.e. first term in { } of (13)) for each element of the wing agree exactly, as they should.

This is indirect evidence that the assumed formula for $\mathbf{F}_s$ (eq. (1)) gives a correct result when integrated in a case of periodic motion. The agreement between the formulae for the power computed by the two different methods corresponds, in the case of a simple lifting airfoil of finite span, to the agreement between the work done against induced drag, and the increase in kinetic energy of the trailing vortex sheet.

* By this is meant only the *mechanical* power (force × distance/time) expended by the insect. There is, of course, a much larger power loss in heating and chemical changes within the insect, as is true for any thermodynamic engine. No account is taken of this here.

In practice errors would be introduced by the fact that v and w are not constant in space and time, but the assumption of their constancy is a standard one in aerodynamic practice, and the error committed is not large.

The integration of eq. (7) can usually be carried out exactly, and by replacing the total force by $(T^2+L^2)^{\frac{1}{2}}$ one can determine $\overline{(C_L^2+C_D^2)^{\frac{1}{2}}}$. The result so obtained is a lower limit to the value of $(\bar{C}_L^2+\bar{C}_D^2)^{\frac{1}{2}}$, since the integration effectively adds all the force elements in the same direction. One thus obtains

$$(\bar{C}_L^2+\bar{C}_D^2)^{\frac{1}{2}}_{\min.}=(L^2+T^2)^{\frac{1}{2}}/(\tfrac{1}{2})\rho\iint_{r,t}2c(r)(W_m^2+W_n^2)\,dr\,dt. \tag{14}$$

The explicit integration of eqs. (10), (11), (13) and (14) is tedious and the results somewhat lengthy. The steps will not be given here, but only an outline of the necessary additional assumptions and approximations. Eq. (14) can be integrated exactly.

The beating cycle is divided into two parts, the down beat, subscript 1, and the up beat, subscript 2. This was done since fairly constant conditions, particularly as to velocities, may obtain on a down or up beat alone, but not for the entire beating cycle. Hence averaging methods and approximations may be used for a single down or up stroke not valid for the entire cycle. Also, the explanation of some of the phenomena of flapping flight require differentiation between the up and down beat. Over the up or down beat θ, C_L, C_D are assumed to be constant with respect to r and t, and to have the values

$$\left.\begin{aligned} C_{L1}&=\bar{C}_L(1+\xi), & C_{L2}&=\pm\bar{C}_L(1-\xi),\\ C_{D1}&=\bar{C}_D(1+\zeta), & C_{D2}&=\bar{C}_D(1-\zeta),\\ \theta_1&=\bar{\theta}+\delta\theta, & \theta_2&=\bar{\theta}-\delta\theta. \end{aligned}\right\} \tag{15}$$

If P is the period of a wing beat, the subscripts 1 and 2 refer to the time intervals $0<t<\pi/\omega_1$ (down beat) and $\pi/\omega_1<t<P$ (up beat), respectively.

ϕ is assumed to vary sinusoidally over the up or down beat, where

$$\phi=A\sin\omega t,\quad \omega_1=\bar{\omega}(1-\eta),\quad \omega_2=\bar{\omega}(1+\eta),\quad \bar{\omega}=2\pi\nu(1-\eta^2).$$

A is the amplitude of motion in the plane of beating, and $\eta=(a-1)/(a+1)$, where a is the ratio of duration of down beat to up beat. η and $\delta\theta$ are assumed small, but ξ, ζ are not so restricted. The double sign for C_{L_2} takes account of the possibility that leading and trailing edge of the wing, or pressure and suction side, may be interchanged on the up and down beat. This does not affect the sign of C_{D_2}.

The principal difficulty at this point is to obtain a satisfactory approximate expression for the radical $(W_m^2+W_n^2)^{\frac{1}{2}}$; once this is obtained the integration is straightforward. No single approximation could be found which was satisfactory for all values of the ratio of the flapping to linear velocity $(rd\phi/dt)/V$ and the time. This ratio varies from zero to much greater than unity over the length of the wing and period of a wing beat, and becomes both positive and negative.

One method is to replace the variable terms in eqs. (10), (11) and (13) by averages. Two preliminary remarks are first necessary:

(1) If we have a complicated expression to integrate, $\int_0^P f(t)\,dt$, and we can guess in some way an average value $\bar{f}$, then $P\bar{f}$ is a good guess for value of the integral. If circumstances are such that we can guess average values over two parts of the range of integration o to a, and a to P, then the average value for the entire interval is given by

$$(a\bar{f}_{0,a}+(P-a)\bar{f}_{a,p})/P.$$

In what follows, P corresponds to the period of a wing beat and the two intervals to the period of the down and up beat, π/ω_1 and π/ω_2.

(2) Suppose $c(r)$ is a simple function, o at the end of an interval o to R, and positive in between, like a semi-elipse or a rectangle. We wish to integrate an expression of the form

$$I=\int_0^R (a_0c(r)+a_1rc(r)+a_2r^2c(r)+a_3r^3c(r)+\ldots)\,dr. \tag{16}$$

The a's are assumed such that the expression converges. Now if S is the area $\int_0^R c(r)\,dr$, I can be written

$$I=a_0S+a_1\bar{r}S+a_2\overline{r^2}S+a_3\overline{r^3}S+\ldots. \tag{17}$$

Now for a simple geometrical figure as above $\bar{r}, \overline{r^{2\frac{1}{2}}}, \overline{r^{3\frac{1}{2}}}$, etc., are all not so very different and one could approximate the desired integral by substituting a single average value for r for all those appearing, that is

$$I\simeq a_0S+a_1\overline{r^{2\frac{1}{2}}}S+a_2\overline{r^2}S+a_3\overline{r^{2\frac{1}{2}}}S+\ldots. \tag{18}$$

The best choice would be the one for which the corresponding a was the largest. In the above case presumably a_2 was the dominant coefficient.

To return to the integration of eqs. (10), (11) and (13). These are simply integrated by replacing the various variable terms by shrewdly chosen averages, the averages being chosen over two stretches as above, the down and up beat. The choice for average values is made in the following way. $(W_m^2+W_n^2)^{\frac{1}{2}}$ was expanded under the two separate assumptions that the ratio $(rd\phi/dt)/V$ was (1) small, and (2) large, and the resulting expressions could then be integrated. In this way expressions corresponding to (16) above were obtained in which the coefficients $a_0, a_1, a_2, a_3,\ldots$ were constant or easily integrated trigonometrical functions of time. Average values were fixed for $r(\overline{r^{2\frac{1}{2}}}$ in (10) and (11), $\overline{r^{3\frac{1}{2}}}$ in (13)) which fitted both approximations moderately well. Also averages for $\cos\phi$, $\sin\phi$, $\cos\omega t$ were chosen which fitted both approximations moderately well. (Both expansions (1) and (2) above are still fairly good when $(rd\phi/dt)/V\sim 1$.) These selected averages were then substituted directly into the original eqs. (10), (11) and (13). As will be seen by comparing the equations below with eqs. (10), (11) and (13), this process simply amounts to replacing all the terms in eqs. (10), (11) and (13) by shrewdly chosen constant values and integrating in two steps. When this is done the results are as given below. S is the total area of both wings. Let the average velocity components in parentheses in eqs. (10) be indicated by

$$W_{sx}=\overline{\omega\sin\phi\cos\theta+V\sin\phi\sin\theta},\quad W_{sy}=\overline{(rd\phi/dt)\sin\theta-\omega\cos\phi},$$
$$W_{sz}=\overline{V\cos\phi+(rd\phi/dt)\cos\theta},$$

and similarly for W_{px}, W_{py}, W_{pz} in eq. (11). $\bar{r}$, $\overline{r^2}$, $\overline{r^3}$ were determined by numerical integration over the wing form of each insect. Eq. (10) gives

$$\left.\begin{aligned}
&\bar{X}_s = 0,\\
&\bar{Y}_s = \bar{C}_L\{(\tfrac{1}{2})\rho\nu S[(\pi/\omega_1)(1+\xi)(W_{m_1}^2+W_{n_1}^2)^{\frac{1}{2}}W_{sy_1}\\
&\qquad \pm(\pi/\omega_2)(1-\xi)(W_{m_2}^2+W_{n_2}^2)^{\frac{1}{2}}W_{sy_2}]\}\\
&\qquad = \bar{C}_L A\\
&\bar{Z}_s = \bar{C}_L\{(\tfrac{1}{2})\rho\nu S[(\pi/\omega_1)(1+\xi)(W_{m_1}^2+W_{n_1}^2)^{\frac{1}{2}}W_{sz_1}\\
&\qquad \pm(\pi/\omega_2)(1-\xi)(W_{m_2}^2+W_{n_2}^2)^{\frac{1}{2}}W_{sz_2}]\}\\
&\qquad = \bar{C}_L B.
\end{aligned}\right\} \tag{19}$$

Eq. (11) gives

$$\left.\begin{aligned}
&\bar{X}_p = 0,\\
&\bar{Y}_p = -\bar{C}_D\{(\tfrac{1}{2})\rho\nu S[(\pi/\omega_1)(1+\zeta)(W_{m_1}^2+W_{n_1}^2)^{\frac{1}{2}}W_{py_1}\\
&\qquad +(1-\zeta)(\pi/\omega_2)(W_{m_2}^2+W_{n_2}^2)^{\frac{1}{2}}W_{py_2}]\},\\
&\qquad = \bar{C}_D C\\
&\bar{Z}_p = -\bar{C}_D\{(\tfrac{1}{2})\rho\nu S[(\pi/\omega_1)(1+\zeta)(W_{m_1}^2+W_{n_1}^2)^{\frac{1}{2}}W_{pz_1}\\
&\qquad +(\pi/\omega_2)(1-\zeta)(W_{m_2}^2+W_{n_2}^2)^{\frac{1}{2}}W_{pz_2}]\}\\
&\qquad = \bar{C}_D D.
\end{aligned}\right\} \tag{20}$$

The quantities appearing in these two equations are defined as follows:

$$\left.\begin{aligned}
&W_{m_1} = (\overline{r^{2\frac{1}{2}}}A\omega_1/2^{\frac{1}{2}}) + (V\cos\theta_1 - w\sin\theta_1)J_0(A),\\
&W_{n_1} = V\sin\theta_1 + w\cos\theta_1,\\
&W_{m_2} = (-\overline{r^{2\frac{1}{2}}}A\omega_2/2^{\frac{1}{2}}) + (V\cos\theta_2 - w\sin\theta_2)J_0(A),\\
&W_{n_2} = V\sin\theta_2 + w\cos\theta_2,\\
&W_{sy_1} = (\overline{r^{2\frac{1}{2}}}A\omega_1/2^{\frac{1}{2}})\sin\theta_1 - wJ_0(A),\\
&W_{sy_2} = (-\overline{r_2^{\frac{1}{2}}}A\omega_2/2^{\frac{1}{2}})\sin\theta_2 - wJ_0(A),\\
&W_{sz_1} = (\overline{r^{2\frac{1}{2}}}A\omega_1/2^{\frac{1}{2}})\cos\theta_1 + VJ_0(A),\\
&W_{sz_2} = (-\overline{r^{2\frac{1}{2}}}A\omega_2/2^{\frac{1}{2}})\cos\theta_2 + VJ_0(A),\\
&W_{py_1} = (\overline{r^{2\frac{1}{2}}}A\omega_1/2^{\frac{1}{2}})J_0(A)\cos\theta_1 + V\sin^2\theta_1\\
&\qquad + VJ_0^2(A)\cos^2\theta_1 + w(16J_1^2(A)/\pi^2)\sin\theta_1\cos\theta_1,\\
&W_{py_2} = (-\overline{r^{2\frac{1}{2}}}A\omega_2/2^{\frac{1}{2}})J_0(A)\cos\theta_2 + V\sin^2\theta_2\\
&\qquad + VJ_0^2(A)\cos^2\theta_2 + w(16J_1^2(A)/\pi^2)\sin\theta_2\cos\theta_2,\\
&W_{pz_1} = (-\overline{r^{2\frac{1}{2}}}A\omega_1/2^{\frac{1}{2}})\sin\theta_1 + w\cos^2\theta_1\\
&\qquad + wJ_0^2(A)\sin^2\theta_1 + V(16J_1^2(A)/\pi^2)\sin\theta_1\cos\theta_1,\\
&W_{pz_2} = (+\overline{r^{2\frac{1}{2}}}A\omega_2/2^{\frac{1}{2}})\sin\theta_2 + w\cos^2\theta_2\\
&\qquad + wJ_0^2(A)\sin^2\theta_2 + V(16J_1^2(A)/\pi^2)\sin\theta_2\cos\theta_2.
\end{aligned}\right\} \tag{21}$$

$J_0(A)$ and $J_1(A)$ are Bessel's function of the first kind obtained from the expansion of $\cos\phi$, $\sin\phi$, where $\phi = A\sin\omega t$.

If the expression in braces in eqs. (19) and (20) is abbreviated by A, B, C and D one imposes the condition that the sum of the horizontal forces $\overline{Y}_p+\overline{Y}_s$ provide the required thrust $T=(\frac{1}{2})\rho C_{Db} S_b V^2$ and that the total vertical forces $\overline{Z}_s+\overline{Z}_p$ provides the lift $L=Mg$, then

$$T=\bar{C}_L A+\bar{C}_D C, \quad L=\bar{C}_L B+\bar{C}_D D. \tag{22}$$

Then by Cramer's rule,

$$\left.\begin{aligned}\bar{C}_L&=\begin{vmatrix}T & C\\ L & D\end{vmatrix}\Big/\begin{vmatrix}A & C\\ B & D\end{vmatrix},\\ \bar{C}_D&=\begin{vmatrix}A & T\\ B & L\end{vmatrix}\Big/\begin{vmatrix}A & C\\ B & D\end{vmatrix}.\end{aligned}\right\} \tag{23}$$

This gives, using the substitution indicated for A, B, C, D, the expression for the average lift and drag coefficients $\bar{C}_L$ and $\bar{C}_D$ in terms of the observed flight parameter for any given insect.

In a similar manner one finds that the total power is

$$\left.\begin{aligned}P=&w\overline{Z}_s+V\overline{Y}_s\\ &+\bar{C}_D\{(\tfrac{1}{2})\rho\nu S[(\pi/\omega_1)(1+\zeta)(W_{m_1}^2+W_{n_1}^2)^{\frac{1}{2}}Q_1\\ &\qquad+(\pi/\omega_2)(1-\zeta)(W_{m_2}^2+W_{n_2}^2)^{\frac{1}{2}}Q_2],\\ Q_1=&\overline{r^{3\frac{1}{2}}}A\omega_1(\tfrac{4}{3}\pi)^{\frac{1}{2}}[\overline{r^{3\frac{1}{2}}}A\omega_1(\tfrac{4}{3}\pi)^{\frac{1}{2}}+(V\cos\theta_1-w\sin\theta_1)\,0{\cdot}8J_0(A)],\\ Q_2=&-\overline{r^{3\frac{1}{2}}}A\omega_2(\tfrac{4}{3}\pi)^{\frac{1}{2}}[-\overline{r^{3\frac{1}{2}}}A\omega_2(\tfrac{4}{3}\pi)^{\frac{1}{2}}+(V\cos\theta_2-w\sin\theta_2)\,0{\cdot}8J_0(A)],\\ W_{m_1}=&\overline{r^{3\frac{1}{2}}}A\omega_1(\tfrac{4}{3}\pi)^{\frac{1}{2}}+(V\cos\theta_1-w\sin\theta_1)\,0{\cdot}8J_0(A),\\ W_{m_2}=&-\overline{r^{3\frac{1}{2}}}A\omega_2(\tfrac{4}{3}\pi)^{\frac{1}{2}}+(V\cos\theta_2-w\sin\theta_2)\,0{\cdot}8J_0(A),\\ W_{n_1}=&V\sin\theta_1+w\cos\theta_1,\\ W_{n_2}=&V\sin\theta_2+w\cos\theta_2.\end{aligned}\right\} \tag{24}$$

Notice that these are not the same averages as for eqs. (10) and (11). Cubic terms are more important in the power, square terms in the forces. The averages for r and the trigonometrical functions are chosen accordingly.

$\bar{C}_D$ and $\overline{Y}_s$, $\overline{Z}_s$ in (24) are computed from the eqs. (23) and (19).

Eq. (14) for the minimum total force coefficient can be integrated exactly. The result is

$$\left.\begin{aligned}(\bar{C}_L^2+\bar{C}_D^2)^{\frac{1}{2}}_{\min.}=&(L^2+T^2)/\{(\tfrac{1}{2})\rho S\nu[(\pi/\omega_1)(W_{m_1}^2+W_{n_1}^2)\\ &+(\pi/\omega_2)(W_{m_2}^2+W_{n_2}^2)\},\\ W_{m_1}^2=&\overline{r^2}A^2\omega_1^2/2+2(V\cos\theta_1-w\sin\theta_1)\,\bar{r}A\omega_1(2/\pi)(J_0(A)+(\tfrac{2}{3})J_2(A))\\ &+(V\cos\theta_1-w\sin\theta_1)^2(J_0^2(A)+2J_2^2(A)),\\ W_{m_2}^2=&\overline{r^2}A\omega_2^2/2-2(V\cos\theta_2-w\sin\theta_2)\,\bar{r}A\omega_2(2/\pi)(J_0(A)+2J_2(A)/3)\\ &+(V\cos\theta_2-w\sin\theta_2)^2(J_0^2(A)+2J_2^2(A)),\\ W_{n_1}^2=&(V\sin\theta_1+w\cos\theta_1)^2,\\ W_{n_2}^2=&(V\sin\theta_2+w\cos\theta_2)^2.\end{aligned}\right\} \tag{25}$$

Note that this is again a different set of values for W_m and W_n, which here could be integrated exactly.

A second method of integrating (10), (11) and (14) replaced $(W_m^2+W_n^2)^{\frac{1}{2}}$ by the sum of the absolute values of W_m and W_n, times a correction factor k which was a very slowly varying function of the ratio W_m/W_n, taken as constant. Thus

$$(W_m^2+W_n^2)^{\frac{1}{2}}=(|W_m|+|W_n|)k.$$

From that step on the resulting integrations could be carried out almost exactly. The resulting formulae are exceedingly long, since a different set of formulae is required for each of the different sign possibilities for W_m, W_n and C_L on the up and down beat.

This second method has the advantage of showing analytically and directly the effect on the vertical force, thrust and power, of varying the flight parameters C_L, C_D, θ and ω on the up and down beat. The effect of independent variations appear as linear terms in ξ, ζ, η and $\delta\theta$, while the interactions appear as cross-products of ξ, ζ, η and $\delta\theta$.

Check computations by the two methods gave results in fair agreement. All of the numerical results given in this paper were obtained by the first method.

DETERMINATION OF FORCE COEFFICIENTS

Applications of eq. (23) to determine C_L and C_D for the twenty-five insects given in Table 1 were made under two assumptions: $\xi=\zeta=0$, or equal force coefficients on the up and down beat, and $\xi=\zeta=1$, or zero force coefficients on the up beat. It was felt that these represented the two limiting cases between which the actual practice must lie. The results are shown in Figs. 2 and 3, which give the average force coefficients for the entire wing beat or the value on the down beat, respectively. The length of the horizontal line represents the range of solution as the drag coefficient of the body varies between zero and unity, the points being plotted for zero-body drag coefficient.

It will be observed that for the case of zero force coefficients on the up beat the ratio of lift to drag does not exceed 3 or 4 to 1, which is quite acceptable aerodynamically. Hence large lift coefficients are always associated with large drag coefficients. All coefficients are positive, the largest lift and drag coefficients being 5·4 and 2·6, respectively. In the case of equal-force coefficients on the up and down beat, there are two instances of negative lift and drag coefficients, and six with zero or negative drag coefficient. The largest lift and drag coefficients are 11 and 20, respectively.

Under both assumptions, therefore, somewhat larger force coefficients are derived than are met with in conventional aerodynamics, but the assumption of equal-force coefficients on up and down beat leads to the additional anomalies of zero or negative drag and lift coefficients. It is, therefore, concluded that the assumption of zero force coefficients on the up beat must represent a much closer approximation to the truth than equal force coefficients on the up and down beat. In other words, the insect derives the great majority of the useful flying force on the down beat (Guidi, 1938; Holst & Kuckemann, 1942). This conclusion is reinforced by comparing points for the same insect on Figs. 2 and 3. The required lift coefficient for forces acting on the down beat alone is in most cases only slightly greater instead of twice

Table 1. *Dimensions and performance data on insects*

Name of insect	M Mass (mg.)	S Total wing area (mm.^{2})	S_b Body cross-section (mm.^{2})	R Length of longer wing (mm.)	ν Frequency of beating (sec.$^{-1}$)	V Velocity of flight (m./sec.)	a Ratio time of lowering to raising	2ψ Projected double amplitude xz plane (degrees)	A Adopted constant amplitude (radians)	Length of body (mm.)	θ Adopted inclination of plane of beating (degrees)
DIPTERA:											
Tabinus bovinus	276	184	63	15·5	96	4	1·5	90	—	23	(30)
Sarcophaga carnaria L.	45	36	12	7·0	160	2	1·5	75	—	12	(30)
Musca domestica	12	20	4·5	5·5	190	2	1·7	90	—	6·5	(30)
Volucella pellucens Meig.	73	78	39	12	120	3·5	1·2	75	—	13·5	(30)
HYMENOPTERA:											
Xylocope violacea	614	172	47	18	130	4	1·3	—	(1·0)	22	(30)
Bombus terrestris Fabr.	388	142	74	16	130	3	1·1	—	(1·0)	19·5	(30)
Vespa germanica	187	98	29	14	110	2·5	1·3	90	—	18	(30)
Vespa crabro L.	567	260	100	22·5	100	6	1·8	50	—	34	(30)
Apis mellifica L.	78	42	27	8·5	250	2·5	1·3	—	(1·0)	13	(30)
Amonophila sabulosa V. del	45	42	82	9·0	120	1·5	1·2	70	—	18	(30)
LEPIDOPTERA:											
Papilio podalirius	300	3600	52	37	10	3·5	—	140	—	25	(60)
Vanessa atalanta L.	134	1080	31	27	10	4	—	150	—	18	(60)
Pieris brassica L.	127	1840	35	31	12	2·5	—	—	(1·2)	23	(60)
Macroglossa stellatorum L.	345	400	68	20	85	5	1·3	80	—	28	(60)
Plusia gamma L.	144	440	36	18	48	1·5	1·1	120	—	19·5	(30)
		mem. m.+ely.									
COLEOPTERA:											
Melontha vulgaris Fabr.	961	402 642	100	28	46	2·5	1·5	130	—	28	(30)
Cetonia aurata	537	260 370	68	20	86	3	1·3	—	(1·2)	19	(30)
Lucanus corvus	2600	800 1220	250	36	33	1·5	1·0	160	—	54	(30)
Telephorus fuscus	109	116 166	20	12·5	72	0·8	1·4	145	—	16	(30)
				anterior							
NEUROPTERA:											
Brachytron pratense Mull.	557	1200	36	36·5	33	5	1·4	75	—	55	(30)
Calopteryx splendens Harr.	120	850	13	30	16	1·5	1·6	115	—	47	(30)
Pyrosoma minium Harr.	38	355	8	25	27	0·6	1·2	100	—	32	(30)
Panorpa communis L.	30	176	8	14·5	28	0·5	1·6	150	—	17	(30)
Orthetrum caerulescens Fabr.	248	1080	22	32·5	20	4	—	—	(1·0)	42	(60)
Aeschna mixtra Latr.	530	1380	30	39·5	38	7	1·7	70	—	63·5	(60)

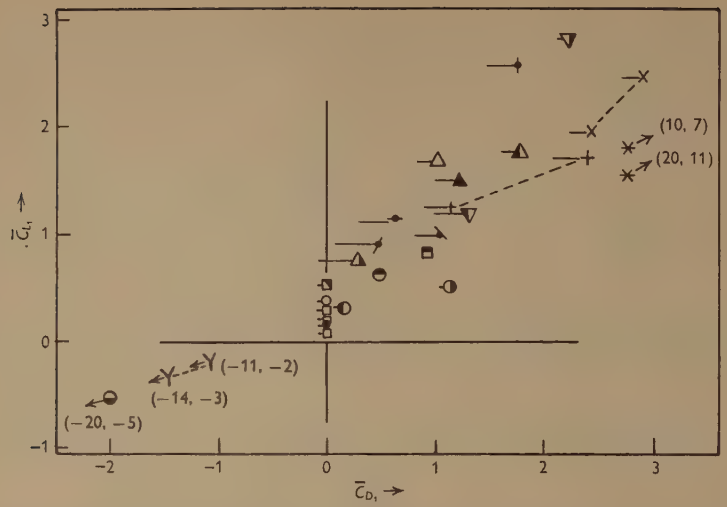

Fig. 2. Average lift *v.* drag coefficient for equal-force coefficients on up and down beat. $\xi=\zeta=0$. Points with arrows fell outside the range of co-ordinates of the figure. The co-ordinates are in parentheses.

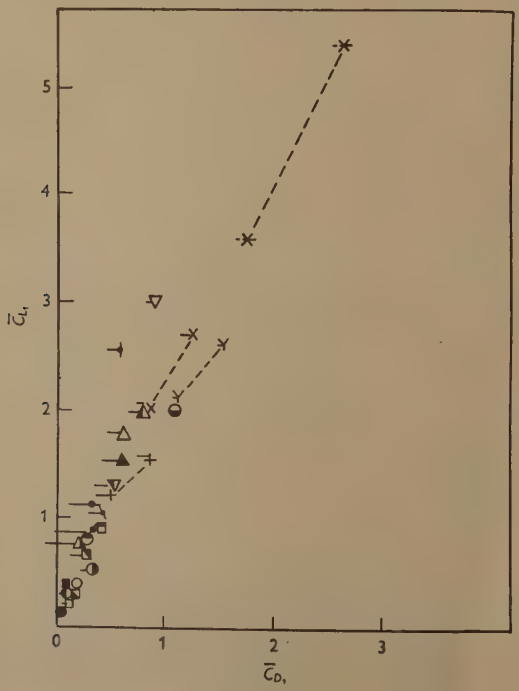

Fig. 3. Average lift *v.* drag coefficient for down beat for zero-force coefficients on up beat, $\xi=\zeta=1$.

as great, as might be expected, than when force acts on the wing during the entire beating cycle. In a few cases, it is less. Except for the 'anomalous cases', the drag coefficient is always a great deal less for the down beat alone than for the entire cycle.

There is a simple explanation for the conclusion that the great majority of the useful flying force must come on the down beat. One draws the components of the average (with respect to r and t) relative wind in the yz plane (Fig. 4). It is specified that the mean flapping velocity $V_F = \overline{(rd\phi/dt)}^{2\frac{1}{2}} = \bar{r}^{2\frac{1}{2}} A\bar{\omega}/2^{\frac{1}{2}}$ is the same order of magnitude as the velocity of flight V, say, within a factor of 3 larger or smaller. This range covers most of the insects considered. Fig. 4 illustrates a number of such diagrams showing various choices (1/3, 1/1, 3/1) for the ratio of flapping velocity to the velocity $\bar{U}$, $V_F/\bar{U}$, and the inclination θ of the plane of beating. Fig. 4 is essentially a geometrical interpretation of eqs. (19) and (20). In this way one can determine to within a quadrant, referred to axes parallel and perpendicular to the relative wind, the direction in which the resultant force $\mathbf{F}_s + \mathbf{F}_p$ on the wing during the up or down beat must lie. Now the average resultant force for both up and down beat *must* provide the necessary vertical force Mg and thrust. This is a force which is predominantly up, with a small forward component. If it turns out, as is usually the case, that no matter how θ, the inclination of the plane of beating is chosen, that for the up beat *any* force within the 90° uncertainty has small or negative components in the direction in which the average resultant *must* lie, then *any* force acting during the up beat is undesirable. The insect will therefore tilt his wings to achieve as nearly zero force coefficients on the up beat as possible.

If the flapping velocity is larger than the linear velocity and θ is $\sim 30°$ or less (Fig. 4*e*), there is some advantage for the insect to reverse the direction of the lift (C_L force) on the up beat, by interchanging either the leading and trailing edge on the up and down beat or the pressure and suction side. In the calculations this assumption was made in those cases in Fig. 2 for which θ was taken to be 30°—all except the six on the $C_D = 0$ axis. There is some evidence that this 'reversed flapping' actually occurs, judging from the drawings and photographs in the literature. For 'reversed flapping' calculations the negative in the double sign in eqs. (15) and (19) is chosen.

When reversed flapping takes place there is also some advantage to the insect to move his wings in a tilted figure 8, with the steep side on the down beat ($\delta\theta > 0$). In this way the average resultant forces on the up and down beat more nearly approach parallelism, and the resultant on the up beat is more nearly in the desired direction. Observation is in agreement with this (Magnan, 1934; Schröder, 1928).

Fig. 4 also serves to show that the force perpendicular to the relative wind or C_L force is the one which most commonly provides the useful or flight-enabling force. Observations indicate (Guidi, 1939; Kukentahl & Krumbach, 1927–34, pp. 573–5; Magnan, 1935, pp. 61, 97, 104, 112, 114; Marey, 1890) that θ is usually 90° or slightly less when the ratio $\overline{(rd\phi/dt)}^{2\frac{1}{2}}/V = \bar{r}^{2\frac{1}{2}} A\bar{\omega}/2^{\frac{1}{2}} V = V_F/V$ is small and can decrease as this ratio increases. Drawing the relative wind diagrams, as in Fig. 4, will show that the C_L force is more nearly in the direction in which the average resultant must lie; the C_D force is at best usually at right angles to the necessary resultant. For an

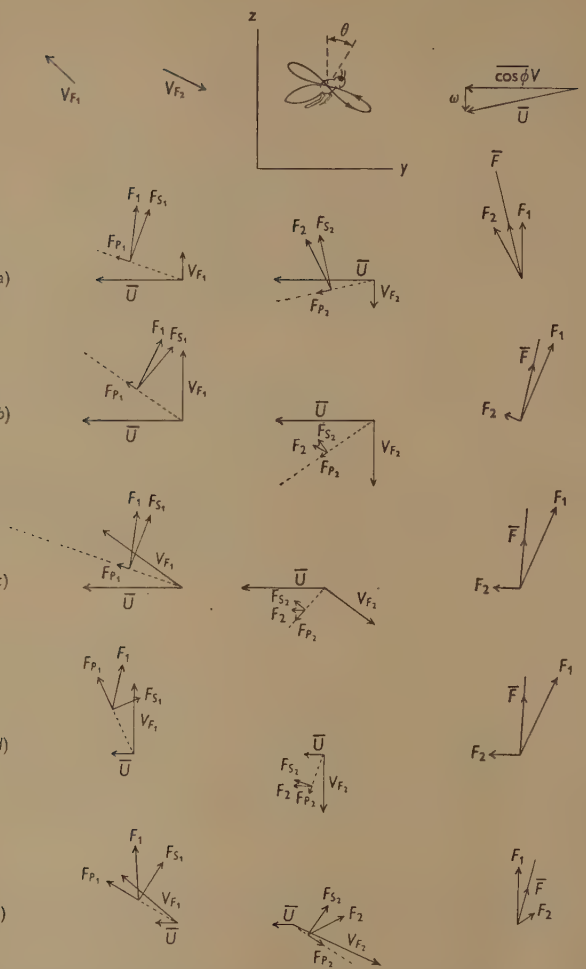

Fig. 4. Graphical determination of forces on up and down beat in YZ plane and average resultant force. V_{F_1} or V_{F_2} = root-mean-square flapping velocity. F_s, F_p = forces perpendicular and parallel to relative wind. F_1, F_2 = average resultant force on down and up beat. F = average of F_1 and F_2. $\overline{U}$ = average velocity of air with respect to insect. The magnitude of the induced velocity w is exaggerated in the vector diagram to the right of the insect. It is always very small compared to at least one of the velocities V_F or U, and is so drawn in (*a*) to (*e*).

(*a*) $V_F \cong 3\overline{U}$, $\theta = 90°$. $\xi = \zeta = 0$. The necessary thrust is not provided unless $F_2 \ll F_1$.
(*b*) $V_F \cong \overline{U}$, $\theta = 90°$.
(*c*) $V_F \cong \overline{U}$, $\theta = 45°$.
(*d*) $V_F \cong \overline{U}/3$, $\theta = 90°$.
(*e*) $V_F \cong \overline{U}/3$, $\theta_1 = 45°$, $\theta_2 = 30°$. Reversed flapping.

exception to this statement, see *d*, Fig. 4, down beat. Here both C_L and C_D forces are nearly equal and equally useful. Thus the insect, in common with all successful flying machines, primarily depends on the force at right angles to the relative wind for flight. Magnan (1934) sketches one exceptional insect, the *Syrphus* fly, for which the C_D force is the useful one. Use of the C_D force is one way to obtain very large force coefficients at the price of considerable power. Other examples of the use of the C_D force will undoubtedly be found when more experimental data is obtained. They will occur when θ is near 90° when hovering, or greater than 90° in forward flight. In

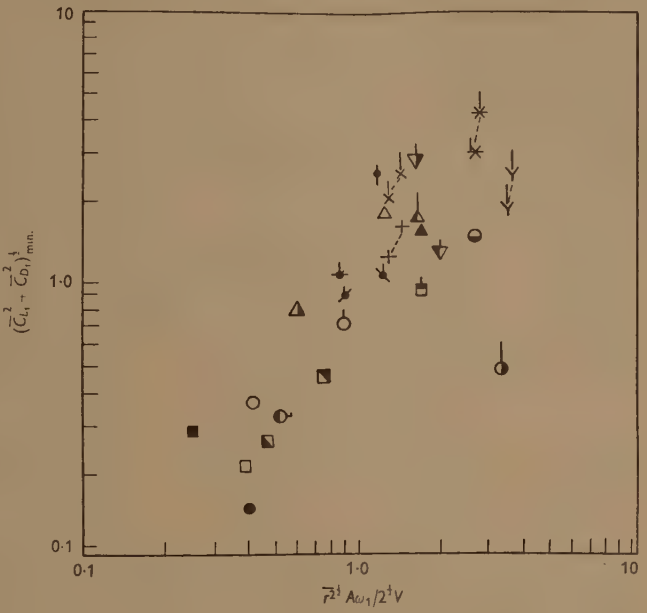

Fig. 5. Minimum total force coefficient on down beat coefficient *v*. ratio of root-mean-square flapping velocity to velocity of flight. $\theta = 30°$, $\xi = \zeta = 1$.

any case the significant force, perpendicular or parallel to the relative wind, can always be determined for any given mode of flight by drawing the relative wind diagram as in Fig. 4.

There remains to explain the occasional large lift and drag coefficients which appear in Figs. 2 and 3. To this end reference is made to Figs. 5 and 6 in which the minimum total force coefficient, determined from eq. (25), is plotted against the ratio of the root-mean-square (with respect to r and t) flapping velocity to the velocity of flight. This ratio measures the importance of the acceleration or inertia forces; when small the inertia forces are negligible. It will be observed that there is a distinct correlation between this ratio and the total force coefficient; when this ratio is small the force coefficient does not exceed those values normally expected in aerodynamic practice.

Thus the abnormally large lift coefficients derived by previous investigators must be attributed to acceleration effects. Both theoretically and practically there is no upper limit to the force coefficient referred to the velocity squared if acceleration forces are permitted. Thus for an impulsive start from rest, as in the case of an oar which is jerked, the terminal velocity may be very small and the force large. Hence the force coefficient referred to the square of the average velocity will be very large. This conclusion holds for both potential and non-potential flow, though in the former case the motion must not be perfectly cyclic.

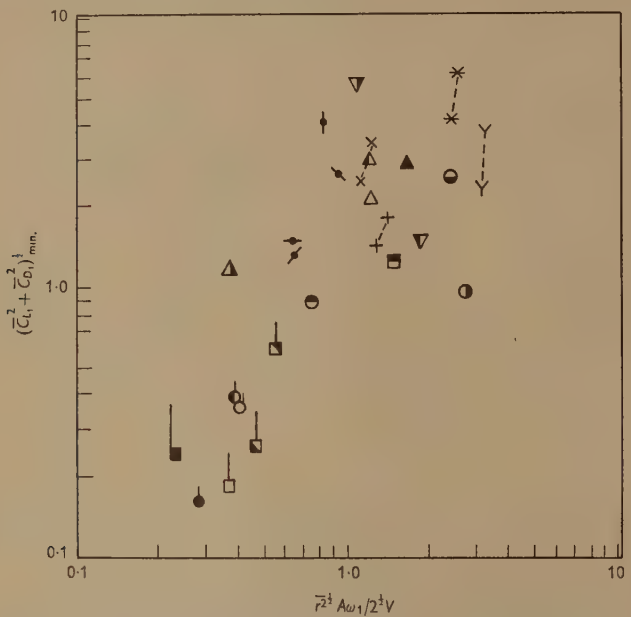

Fig. 6. Minimum total force coefficient on down beat v. ratio of root-mean-square flapping velocity to velocity of flight. $\theta=60°$, $\xi=\zeta=1$.

Part of the high lift coefficient may be due to a slotted wing or flap effect. For those insects with two pairs of wings which overlap, the anterior always overlay the posterior, which is the correct arrangement for a slotted wing. In some cases the posterior is hinged by hooks to the anterior, in which case the posterior may act as a flap.

In Figs. 5 and 6 the vertical lines run up to the values of $(\bar{C}_L^2+\bar{C}_D^2)^{\frac{1}{2}}$, as determined from the separately derived values of $\bar{C}_L$ and $\bar{C}_D$ given in Fig. 3. Computations of $(\bar{C}_L^2+\bar{C}_D^2)^{\frac{1}{2}}_{\min.}$ were also made for the case of equal force coefficients on the up and down beat ($\xi=\zeta=0$) and for assumed $\theta=90°$ and $0°$ (not given here), and all led to essentially the conclusions above. In the case of the remaining figures in this paper,

they all refer to zero-force coefficients on the up beat ($\xi = \zeta = 1$). Calculations were also made for $\xi = \zeta = 0$ but are not given, since they indicated in some cases too small or negative values for the power, corresponding to the zero or negative values of C_D.

Since the observations did not provide values of θ they had to be assumed, though in some cases they could be inferred from the drawings of Magnan. This assumed

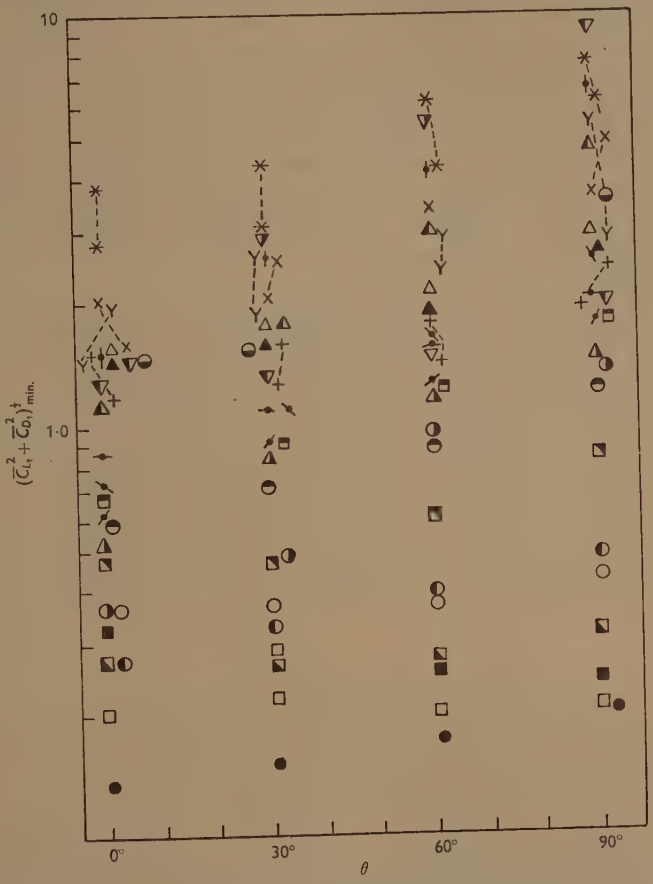

Fig. 7. Minimum total force coefficient on down beat as a function of the assumed inclination θ of the plane of beating. $\xi = \zeta = 1$.

value influenced the value of the double amplitude $2A$ of the wing motion in the plane of beating, since the double amplitude projected on the vertical (xz) plane, 2ψ, was the observed quantity given by Magnan. Hence the smaller the assumed θ, the larger the derived $2A$, which would vary between its projected value and 180° as θ varied from 90° to 0°. The effect of the assumed θ on the derived minimum total

force coefficient is shown in Fig. 7, where it can be observed by tracing out a curve for a single insect that the force coefficient can at most increase by a factor of 4 or 5 to 1 as θ varies from 0 to 90°. The variation is much less for those insects with a projected double amplitude close to 180° and for those insects for which no projected amplitude was given, so that a constant amplitude in the plane of beating had to be assumed. The range of solution for $0 < C_{D^0} < 1$ is not given in Fig. 7; the range was negligible. Assumed values of θ and A in Table 1 are in parentheses.

The actual values of θ as used for the computations of C_L, C_D (Figs. 2 and 3) and the remaining figures involving the power were chosen as indicated in Table 1. In general, for $\overline{(r(d\phi/dt))^2}^{\frac{1}{2}}/V \gtrsim 1$, θ was assumed to be 30°, whereas if this ratio were much less than 1, $\theta = 60°$ was assumed. The values of θ chosen by this rule were the least values compatible with the available drawings of Magnan and others. The consequence of such a choice is that A is a little too large, and the derived values of $\bar{C}_L$, $\bar{C}_D$ and $\bar{C}_D/\bar{C}_L$ and the power a little too small. This is considered to be 'advantageous' to the insect in that the necessary power is a minimum. The range of solution as θ is varied can be estimated from Fig. 6.

From another standpoint also the derived average values of $\bar{C}_L$, $\bar{C}_D$ and $(\bar{C}_L^2 + \bar{C}_D^2)^{\frac{1}{2}}$ are minimum values. Since the actual instantaneous values of these quantities must vary with r and t, at some time the instantaneous values must exceed their averages.

According to Table 1, col. 8, it will be observed that the down beat is slower than the up beat, or $\omega_1 < \omega_2$ (see also Marey, 1895, p. 228; Guidi, 1938, p. 1108). This is an 'advantageous' mode of flight to the insect, in that it tends to reduce the power for fixed frequency of flapping and fixed requirements of vertical force and thrust. Since the down beat *must* provide most of the force, and since the force goes up as the velocity squared of the wing while the power goes up as the velocity cubed, it requires less power for the same average force when a smaller force is exerted over a longer time ($\omega_1 < \omega_2$) than a larger force over a shorter time ($\omega_1 > \omega_2$).

GEOMETRICAL SIMILARITY AND POWER

The degree of geometrical similarity of the insects considered can be examined in Figs. 8 and 9. Here the wing length and area are plotted against the mass. The dotted line in each figure indicates the expected slope for geometrical similarity, i.e. the quantities plotted should be proportional to $M^{\frac{1}{3}}$, $M^{\frac{2}{3}}$ respectively. Geometrical similarity is realized with considerable dispersion except possibly for the wing area, which seems to increase slightly faster than $M^{\frac{2}{3}}$. Fig. 9 also shows that the Lepidoptera and Neuroptera (squares and circles), which tended to have smaller force coefficients, have relatively larger wing areas for their masses.

In contrast to the large individual departures from geometrical similarity for the wing area, the power as a function of the mass shows surprisingly small dispersion (Fig. 10). The power is very nearly proportional to the mass (dotted line indicates proportionality). This proportionality is reasonable from the standpoint of metabolism, and is also in keeping with the observation that the wing area increases slightly faster than $M^{\frac{1}{3}}$. From aerodynamic considerations it can be shown that

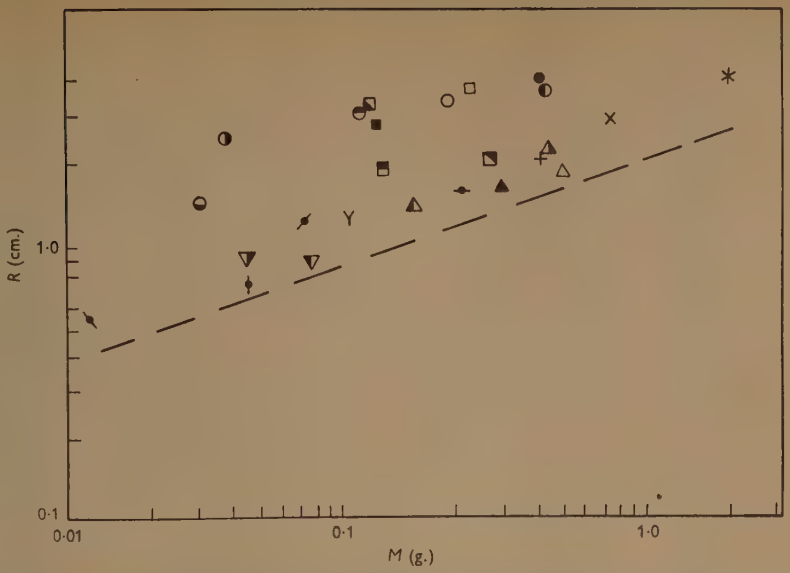

Fig. 8. Wing length v. mass of insect. The dotted line indicates the slope for geometrical similarity, $R \propto M^{\frac{1}{3}}$.

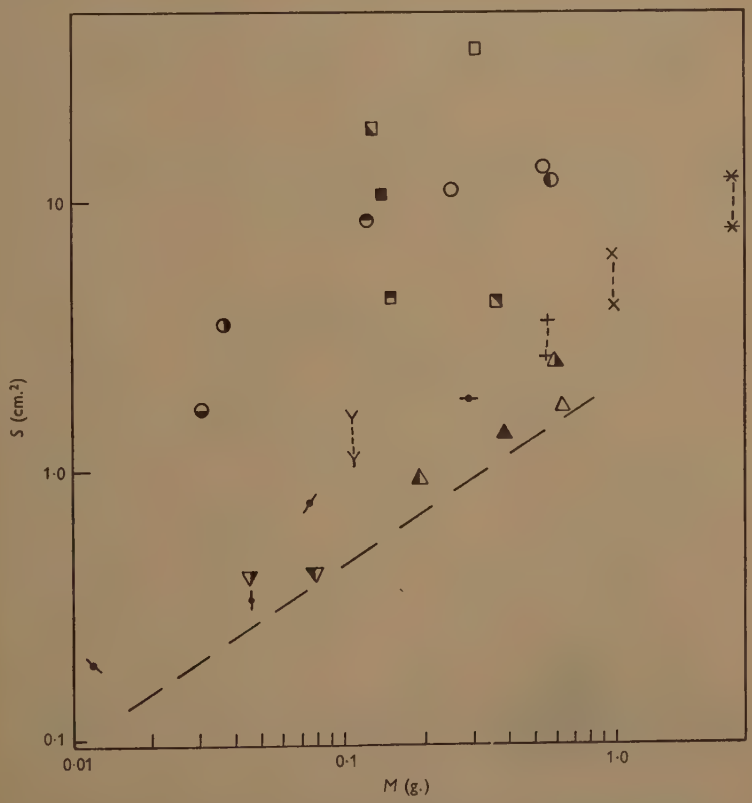

Fig. 9. Wing area v. mass of insect. The dotted line indicates the slope for geometrical similarity, $S \propto M^{\frac{2}{3}}$.

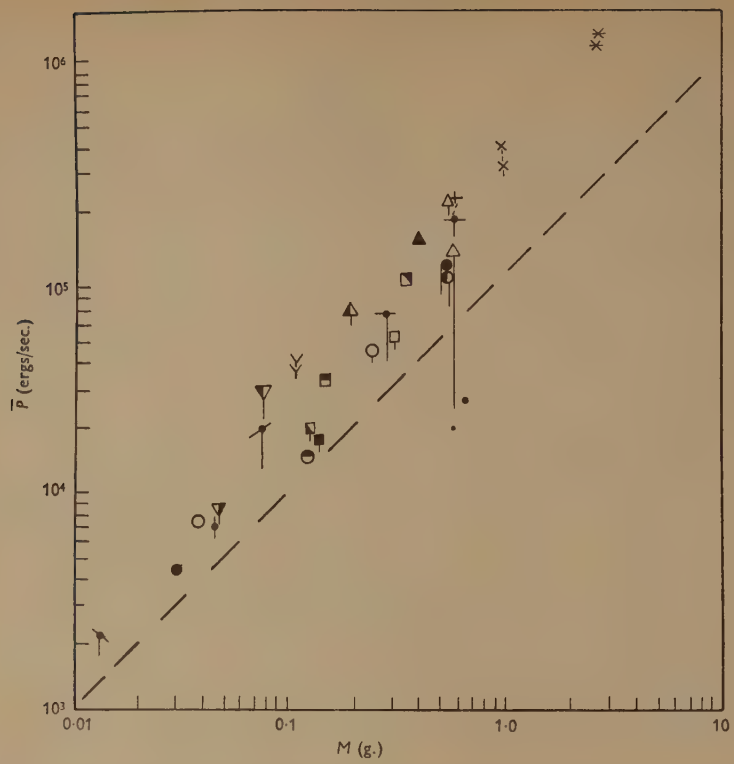

Fig. 10. Power *v.* mass, $\xi=\zeta=1$. The dotted line indicates the slope for simple proportionality.

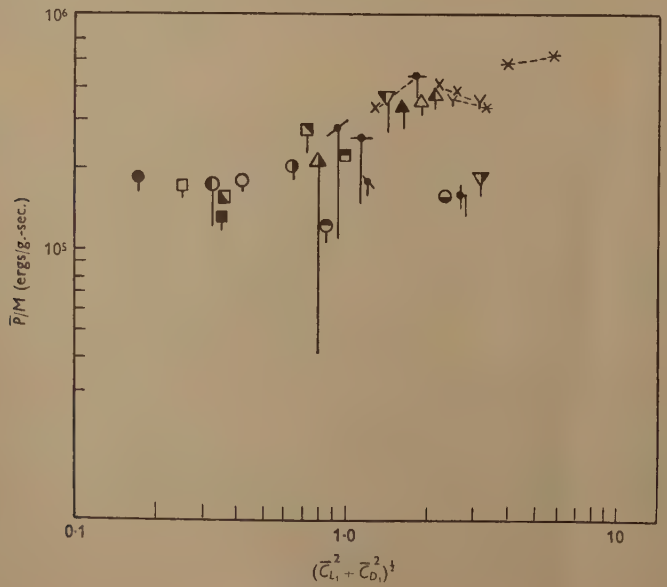

Fig. 11. Power per gram as a function of the total force coefficient. $\xi=\zeta=1$.

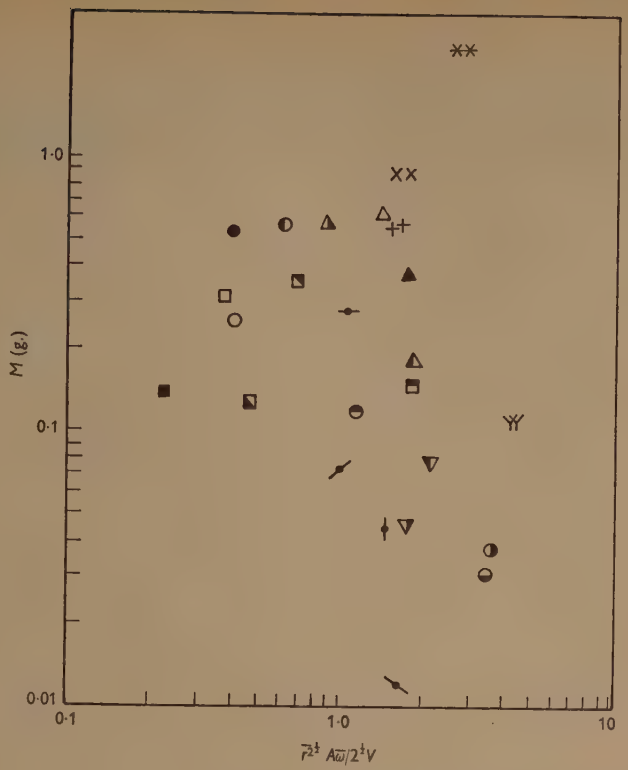

Fig. 12. Mass *v.* ratio of root-mean-square flapping velocity to velocity of flight.

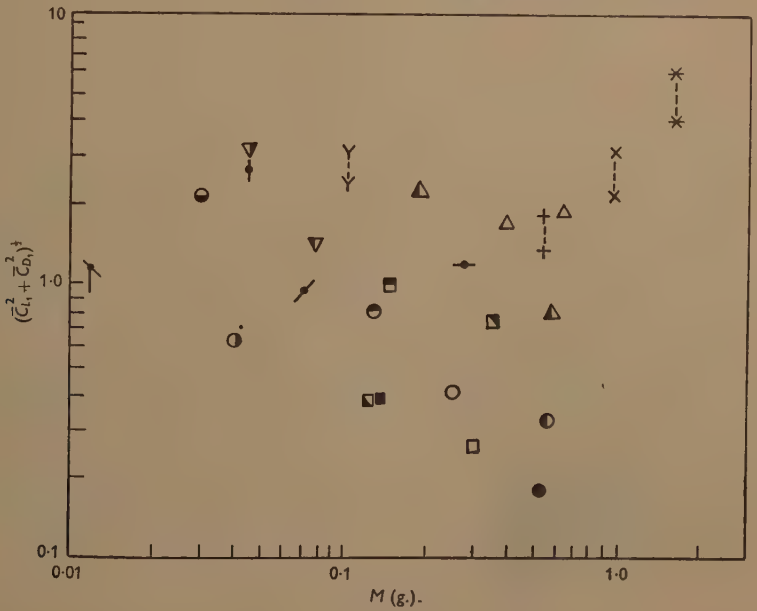

Fig. 13. Total force coefficient on down beat *v.* mass. $\xi = \zeta = 1$.

other things being equal, either the wing area must increase faster than is required by dimensional similarity, the power increase faster than the mass, or both, as seems indicated in the present case. The uncertainty of the data renders this conclusion as yet only tentative.

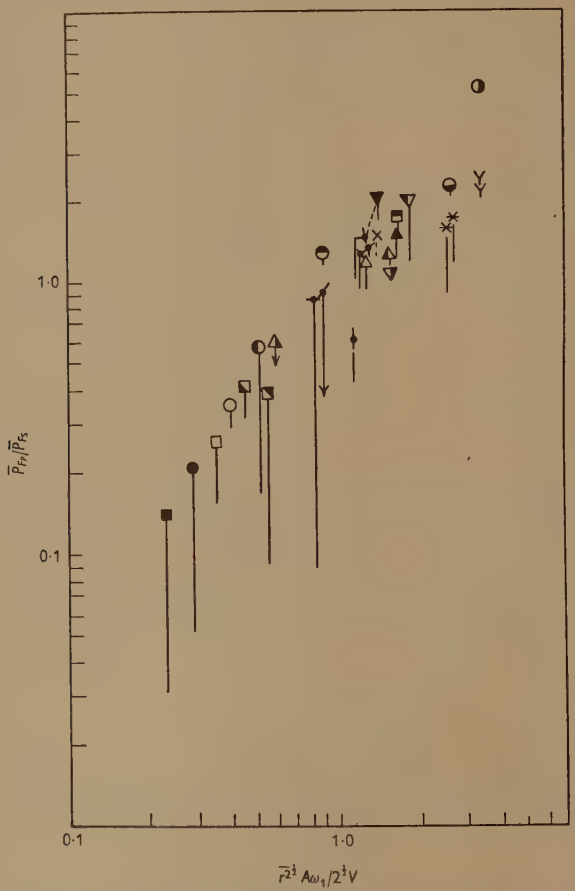

Fig. 14. Ratio of power expended against drag to power expended against lift forces *v.* ratio of root-mean-square flapping velocity to velocity of flight. $\xi = \zeta = 1$.

Fig. 11 gives the specific power, or power per gram, as a function of the total force coefficient. They increase together, again with surprisingly small dispersion. Fig. 11 indicates about 10^{-2} horse-power per lb.—better than a man, scarcely in a class with aircraft engines!

There is a very slight negative correlation of mass with ratio of flapping velocity to velocity of flight (Fig. 12). There is also a slight negative correlation between total

force coefficient and mass (Fig. 13), which does not support Rashevsky's conclusion that C_L should increase with the mass (Rashevsky, 1944). In general, both lift and drag coefficients for insects are large, larger than those for birds, in the few cases where they have been evaluated (Demoll, 1919; Walker, 1925).

In Fig. 14 is plotted the ratio of the power expended against F_p (forces in C_D, second term of eq. (13)) to power expended against F_s (forces in C_L, first term of eq. (13)) as a function of the ratio of the root-mean-square wing-flapping velocity

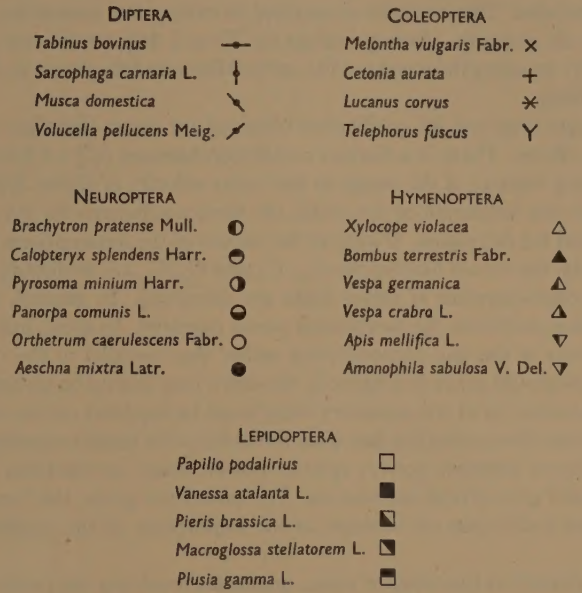

Fig. 15. Identifications of points for all figures.

to the velocity of flight. Fig. 14 indicates that the fraction of the power expended against F_p increases with the importance of the inertia forces. This shows the increasing price in 'wasted power' paid for the use of inertia forces in flight, since C_D forces are usually in an undesirable direction, as Fig. 4 showed.

In Fig. 14 the dispersion is also surprisingly small, in view of the large individual departures from geometrical similarity. The small dispersion of quantities involving the power, as opposed to quantities involving the geometrical dimensions, indicates that as power plants they are essentially similar, despite their great diversity in form.

The range of solution for quantities involving the power (Figs. 10, 11 and 14) as the drag coefficients of the body C_{Db} varies between 0 and 1 is shown by the length of the vertical lines, the points being plotted for $C_{Db}=0$. The dependence of the power on C_{Db} is considerable, a consequence of the dependence of C_D on C_{Db}.

There are four pairs of similar star-type points on the figures, connected by dotted lines. These are the Coleoptera. The upper point of each pair refers to calculations

ignoring the elytra as wings, the lower includes them as a part of the membranous wings. This was done since the contribution of the elytra to flight was not known, but it was felt that these two cases were the limiting ones.

Fig. 15 identifies the points in all the figures, and Table 1 summarizes the pertinent flight data.

SUMMARY

1. General formulae are derived giving the lift, thrust and power when the wing motion is specified. The formulae are applied to twenty-five insects for which quantitative data are available. Average values for lift and drag coefficients, C_L and C_D, are derived by equating the weight to the vertical force and the thrust to the horizontal drag of the body.

2. The large drag and lift coefficients obtained for insect flight are attributed to acceleration effects. There is a distinct correlation between $(C_L^2 + C_D^2)^{\frac{1}{2}}$ and the ratio of the flapping velocity of the wings to the linear velocity of flight. When this ratio and therefore the accelerations are small, the force coefficients do not exceed those to be expected for flat plates. Owing to the nature of the assumptions and approximations made, the values derived for C_D, C_L and C_D/C_L are minimum values.

3. Other characteristics of insect flight are discussed. In general, insects fly in such a way as to minimize the mechanical power required. In most, but not all cases, the useful force is the one perpendicular rather than parallel to the relative wind. The wing tips should move in a figure 8, the down beat should be slower than the up beat, and the majority of the necessary force must be supplied on the down beat.

4. Figures are given using the data from the twenty-five insects considered, showing average relations between power, specific power, mass, acceleration forces, force coefficients and geometrical dimensions. The power per gram, the 'wasted power', and the force coefficients all increase as the importance of the acceleration forces increases.

5. When plotted as functions of mass, quantities involving the power show much less dispersion than quantities involving the geometrical dimensions. This is taken to mean that despite the diversity of insect form, as 'power plants', they are all essentially similar.

6. A table of the observed or adopted flight parameters (frequency of beating, mass, wing area, velocity of flight, amplitude and orientation of wing motion) is appended.

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